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THE REACTIONS OF SPONGES, WITH A CONSIDERATION OF THE ORIGIN OF THE NERVOUS SYSTEM

BY

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WITH THREE FIGURES

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I. INTRODUCTION

Previous attempts at the discovery of the nervous system of sponges have been made almost exclusively from an anatomical standpoint and with such negative results that Vosmær and Pekel-

haring ('98, p. 18) believed themselves justified in declaring that the cells of sponges "are not connected in a way so as to enable them to conduct stimuli from one cell to another" and that these animals are therefore "destitue of the principle, the significance of which culminates in nervous tissue." It was the chief purpose of the investigations recorded in the present paper to ascertain whether there was any *physiological* ground for the assumption that sponges possess a nervous system, or whether from the standpoint of their activities, as well as of their structure, they showed no evidence of nervous organs. The general inertness of sponges has doubtless long deterred investigators from attempting a study of their reactions, and it must be confessed that even on close examination they show only a few form of inconspicuous response These few types of movement, however, are of considerable interest, for, as the following account will show, they throw considerable light not only on the question of the nervous system, in sponges but also on the still more fundamental problem of the origin of the nervous system in general.

The species on which my work was done was Stylotella heliophila Wilson, a monaxonid demosponge belonging to the order Helichondrina. This species will be described in a monograph on the sponges of Beaufort, N. C., soon to be published by Dr. H. V. Wilson, and I am indebted to Dr. Wilson for having called my attention to this sponge, which in all respects was extremely satisfactory for the work I had planned. My investigations were carried out in June and July at the Beaufort Laboratory of the United States Bureau of Fisheries, and I am under obligations to Commissioner G. M. Bowers for the privilege of working at this laboratory and to its director, Mr. H. D. Aller, for generous provision during my stay there.

2. STYLOTELLA UNDER NATURAL CONDITIONS

Stylotella heliophila is found in great abundance in the shallow water near the Beaufort Laboratory. It grows in masses about as large as a double fist and is attached to stones, oyster shells, and like materials. It is dirty orange-yellow or greenish yellow

in color and, though sometimes simply massive in habit, it generally rises in finger-like processes from an incrusting base (Fig. 1). It is found near low-water level, and some colonies are so situated that at the spring tides they may be continuously exposed to the air for as long as four hours. As a rule the massive form is characteristic of those colonies which from time to time are exposed to the air; the long-fingered type is limited almost exclusively to such as are never uncovered by the sea even at the lowest tides. A Stylotella grows on the upper surfaces of stones, etc., in shallow

Fig. 1. Side view of a colony of Stylotella heliophila Wilson, about natural size. From a photograph taken by Dr. H. V. Wilson.

water, it is often in strong sunlight for the greater part of the day and in fact when uncovered by the tide it may also be exposed to the extreme heat of the sun for hours at a time. That it not only survives under such conditions but seems even to court sunshine has doubtless given occasion to its specific name *heliophila*. The water in which it thrives is often deeply laden with sediment and this at times may shield it partly from the sun's rays, but when the water is clear or the sponge is exposed to the air, it receives

the full force of these rays yet without any apparently disastrous effects.

If a colony of Stylotella in natural position in quiet, clear seawater is closely examined, its numerous oscula, which occupy either the tips of the fingers or slight elevations on the surface of its body, will be found as a rule to be widely open, so that an observer can look far down into the interior of the animal and see much of the branched gastral cavity and the excurrent canals leading into it. Although the fingers of the sponge are generally not much over seven to eight millimeters in diameter, the oscula may measure as much as four and a half millimeters in width when fully expanded thus giving a considerable view of the internal cavities. If such a colony is suddenly lifted out of the seawater into the air, the water rapidly drains from it and the air rushes through its oscula into its internal chambers. On returning such a sponge to the sea, the air thus introduced is with difficulty dislodged and may eventually as large bubbles distort and deform the sponge. Sponges that are exposed to the air on the beach by the natural fall of the tide show no such inclusions of air, and an examination of them in seawater brings to light the fact that their oscula are all firmly closed thus preventing the entrance of air. The steps of this closure can be easily followed by watching a sponge that is gradually becoming exposed to air when, in a quiet sea, the tide is falling. Under such circumstance the oscula remain open till they come into direct contact with the air when, with about three minutes, they close. If now the sponge colony is moved into deeper water, the oscula will reopen in from seven to ten minutes. If oscula at different levels on the same sponge are watched, those that come in contact with the air first, close first and those that are situated at a deeper level do not close until they in turn have been exposed to the air. These conditions are easily reproduced in the laboratory. Thus if a colony of fresh sponge is carried into the laboratory and placed in a glass vessel in which a current of seawater in kept running, on exposing the tip of any finger to air its osculum will close in a few minutes to reopen after it has been reimmersed in seawater for about ten minutes. On quickly removing a sponge from the sea the chim-

ney-like membranes around the oscula very generally collapse showing that they are delicate structures. That the normal closure of the osculum is not a purely mechanical collapse of this kind is seen from the fact that when a sponge in which the closure has taken place by gradual exposure to air is returned to seawater, its oscula do not flap open but can be seen to expand only gradually as by the relaxation of a sphincter. It is quite clear that the closure of the osculum is a definite response, which, among other things, prevents the entrance of air into the cavities of the sponge when, by a fall of the tide, the sponge becomes exposed to the air.

Another response which can be observed in Stylotella in its natural state is seen on comparing specimens that have been exposed for some time to the air on the beach with specimens still in the water. The latter as a rule have a plump appearance and a relatively smooth surface, whereas those that have been in the air look somewhat shriveled, and their surfaces are roughened as though their flesh had shrunken down on a rather resistant skeleton. At first sight it would seem that the sponge had shriveled simply because under action of gravity the water had been drained from it, but that this shriveling is probably not thus produced, but is dependent upon a positive contraction of the flesh of the sponge, is seen from the fact that the same shriveled, rugose appearance can be assumed by a sponge *in seawater* under conditions to be described later. These two reactions, the closing and opening of the oscula, and the shriveling and filling out of the common flesh of the body, are the most obvious natural responses exhibited by Stylotella.

3. STRUCTURE OF STYLOTELLA

Stylotella is an encrusting sponge that usually throws up longer or shorter fingers (Fig. 1.) These fingers, which represent the individuals in the colony, may attain a length of four centimeters and each one carries near its distal end usually one, sometimes two or more oscula. When fully expanded the oscula are roundish openings in a dome-shaped elevation or at the end of a more

chimney-like projection. When contracted they are completely closed and the delicate tissue about them is puckered into a slight spine-like elevation, the point of which represents the real position of the osculum. The largest oscula when fully open measure, as already stated, about four and a half millimeters in diameter. From each osculum a branched gastral cavity extends through the substance of the sponge either down the length of a finger or into the massive body, depending upon the position of the osculum. In the fingered forms the gastral cavities lie either near the axis of the fingers, and are thus buried in the substance of the sponge, or on the surface of the finger, in which case they can be

Fig. 2. Radial portion of a transverse section of Stylotella; the flesh of the sponge is tinted, the cavities are untinted; on the extreme left is the dermal membrane pierced by two ostia that lead into a large subdermal cavity, from which incurrent canals lead to the flagellated chambers, which in turn open by an excurrent canal into the gastral cavity at the extreme right. × 25.

traced on the outside as translucent-walled canals well down the length of the finger. Excepting the regions where the gastral cavities show from the outside, the whole external surface of the sponge is faintly rugose and of a dirty-yellow color.

The internal structure of the sponge is well seen in a transverse section of a finger. On the outside of such a section (Fig. 2) is a well defined membrane pierced in many places by dermal pores or ostia. These openings are roundish or oval in outline and, as seen in living bits of membrane torn from the outer surface of the sponge, they measure from ten to twenty micra in diameter. The ostia lead into relatively large sub-dermal cavities, which

often form a definite layer around the whole finger directly under the dermal membrane From these sub-dermal cavities pass off the incurrent canals, which lead centrally into the flagellated chambers. These chambers are usually spherical in form, measuring about twenty to forty micra in diameter and forming a more or less compact layer surrounding the gastral cavity; they connect with this cavity by short, irregularly branched, excurrent canals. Where the gastral cavity is close to the external surface of the sponge, there are apparently few or no ostia, sub-dermal cavities, or flagellated chambers, but the dermal membrane and the lining of the gastral cavity coalesce to form the translucent wall already mentioned. In the living condition of the sponge the layer of flagellated chambers is orange in color while the other parts of the animals are mostly dirty-yellow in tint.

I have not studied with any fulness the histology of stylotella but in good osmic-acid preparations cut into sections ten micra thick and stained in picrocarmine much of the cellular structure of this animal can be made out. The outer surface of Stylotella is covered with a dermal epithelium composed of polygonal cells that are usually extremely thin, though at places they show a condition approximating that of a cuboidal epithelium. The sub-dermal cavities, incurrent and excurrent canals, and gastral cavity are lined with a very flat epithelium, whose presence is difficult to demonstrate unless it is well preserved and cut at a favorable angle. The flagellated chambers are of course lined with a layer of relatively large choanocytes. In some places the dermal membrane seems to be made up of nothing but the dermal epithelium and the lining epithelium of the sub-dermal cavity and is therefore extremely thin. In most regions, however, it contains several other kinds of cells. Of these one set is represented by elongated, spindle-shaped cells, the so-called myocytes, and these are arranged like irregular sphincters around the ostia. They also surround abundantly the sub-dermal cavities, gastral cavity, osculum, etc. Structurally they have the appearance of a primitive kind of smooth muscle-fiber. In some places in my preparations they seem to lie directly on the exposed surfaces of the canals and cavities that they bound as though they were merely elongated

epithelial cells, as in fact they are believed to be in many other sponges (Minchin, '00, p. 46; Schneider, '02, p. 260). Even admitting, however, that in Stylotella they are in all cases covered by an epithelium, the epithelium is certainly in most instances so extremely thin that these cells are almost in contact with the sea-water passing through the cavities that they surround.

In the region of the osculum the myocytes are especially numerous and form a conspicuous sphincter on the inner face of the oscular collar and internal to the mass of longitudinally arranged spicules which surround this opening. As a result the contraction of the osculum is accomplished without much folding of the surface

Fig. 3. Transverse section of the base of an oscular collar of Stylotella. The central cavity is the osculum, which, as is shown on the right is directly surrounded by a sphincter of myocytes, external to which is the tissue containing the spicules. × 35.

of the sponge next the oscular cavity, for this surface is immediately in contact with the contractile material, if in fact it is not contractile itself. On the other hand the outer substance of the oscular membrane and the palisade of spicules are thrown into many folds in contraction as though they passively followed the constricting ring of myocytes to the outside of which they are attached (Fig. 3). This palisade-like arrangement of the rigid siliceous spicules is the only one that would allow an easy contraction and expansion of the osculum and it is in strong contrast with that of the spicules in the rest of the sponge, in which these bodies show no such grouping.

Although the larger apertures, including the osculum, possess well defined sphincters by which they are closed, I have never been able to find in Stylotella systems of radiating fibers by which they might be opened. Now and then I have seen what seemed to be slight radiating systems, but they were always associated with closed or partly closed openings and might perfectly well have owed their origin to the mechanical stretching of the elastic tissue in the neighborhood of a sphincter. I am inclined to believe therefore that the myocytic sphincters in Stylotella work against the general elasticity of the body tissue, which may have a slight radial arrangement in their neighborhood, rather than that they oppose a well defined system of radial myocytes. The absence of radial fibers in many sponges in which sphincters occur has been noted by Minchin ('00, p. 46).

4. REACTIONS OF STYLOTELLA

A. Movements of the Oscula

The opening and closing of the oscula in Stylotella, as already mentioned is the most obvious of the responses of this sponge. If a colony under ordinary conditions is examined, some of the oscula will almost certainly be found closed, though the majority will be widely open. If a small colony is closely inspected under a low power of the microscope, the open oscula will be seen to emit a large number of minute particles indicating that a current is setting out through these openings. In what seem to be closed oscula a minute but otherwise similar current can often be detected showing that they are really not closed. Some oscula, however, show absolutely no current, though I have invariably found that when in such cases the oscular tip was cut off, the current almost instantly could be seen, and I believe, therefore, that the oscula do close completely and thus check absolutely the current that ordinarily passes through them. In order to get some idea of the natural movements of the oscula, a vigorous colony of Stylotella was isolated and three of its oscula were kept under approximately hourly observation for three days. The results of these observations are summarized in Table I.

TABLE 1

Times in hours and minutes during which in the course of three days oscula 1,2, and 3 were open or closed

NUMBER OF THE OSCULUM	TIME IN HOURS AND MINUTES OF EACH SUCCESSIVE PERIOD OF OPEN OR CLOSED STATE								TOTAL TIMES IN 72 HOURS	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Osculum 1.....	0.45	2.00	19.05	3.20	20.15	7.50	2.35	16.10	42.40	29.20
Osculum 2.....	21.50	3.20	24.20	1.30	21.00				67.10	4.50
Osculum 3.....	0.45	0.25	21.40	2.35	23.50	0.15	23.10		68.45	3.15

Since the three oscula whose conditions are recorded in Table 1 were on the same colony and near together and were exposed to almost identical surroundings, the fact that osculum 1 was closed on the average one hour in every two and a half, while oscula 2 and 3 were closed only one hour in every eighteen, must be attributed to the difference in constitution of osculum 1 as contrasted with that of the other two. The condition of general openness as exemplified by oscula 2 and 3 is doubtless typical for these organs. At least in any vigorous sponge under normal conditions, the majority of the oscula will be found open much of the time. When an osculum opens or closes, it does so in response to some stimulus. To ascertain what the effective stimuli are in this form of response, I have studied the ocular reaction in relation to mechanical and chemical stimulation and to heat and light.

a. Mechanical Stimulation

When at low tide a specimen of Stylotella was transferred from the shallow water of the outside to the laboratory tank, an operation that required about ten minutes, it was found that the anima that in the outside water had most of its oscula open usually had the majority of them closed when it had arrived in the laboratory, notwithstanding the fact that it had not once been exposed to the air during this transfer. At first it was suspected that this closure of the oscula was due to the disturbance caused in loosening the sponge from the bottom, etc., but it was found that this was not

so, for, if a sponge after it has been dislodged, is left in the outside seawater, its oscula remain open. Change in illumination was also suspected of being the cause of the contraction. But if a sponge in its natural situation in full sunlight is suddenly shaded to an extent not unlike the diffuse daylight of the laboratory, the oscula still remain open. The reduction in the intensity of the light, then, is not the cause of the contraction. After this the effects of currents was tried, for, so far as could be judged, the chief difference between the condition of the sponge in its usual habitat and in the laboratory, aside from recent disturbance and illumination, was that in the first situation it was in moving seawater and in the second it was in the same water standing still. An aquarium with a free circulation of water was set up and sponges were placed in this in such situations that they caught the full effects of the current. The results were very uniform. Sponges from the exterior often arrived in the laboratory with many of their oscula closed. On putting these specimens into the aquarium under a strong current of seawater they almost invariably opened their oscula within ten minutes. The following record from my laboratory note-book will give a good idea of the character of these changes.

12:40 p.m. A sponge brought directly from the outside was placed in the aquarium with a strong circulation of seawater. Many of the oscula were closed.

12:45. Oscula began opening.

1:10. All oscula have been widely open for some time. Seawater current is now cut off.

1:12. Many oscula are closing.

1:14. Most oscula are closed. Seawater current is now turned on again.

1:18. Oscula have begun opening.

1:25. Most oscula are open.

1:39. Oscula remain open.

This and many other similar experiments pointed to the importance of currents in keeping the oscula open, but this form of experiment did not show what particular aspect of the current caused the osculum to open or to remain open. Did the sponge

give out excretions which in quiet water gathered to such an extent in its immediate neighborhood as to cause its oscula to close and only on the removal of these by a current of water would the oscula open, or did the current carry oxygen to the sponge or act in a purely mechanical way to induce the opening of the oscula? To test these matters the following simple apparatus was constructed. Three cylindrical glass aquaria of considerable size were placed at three levels so that the water from the uppermost aquarium could be siphoned freely into the intermediate one from which the water overflowed into the third. Having filled the apparatus with seawater, it was possible to keep it running continuously with the *same* seawater by returning that which collected in the third or lowest aquarium to the uppermost one. If now the current of seawater carried away excretions from the sponge or brought oxygen to it and these operations had anything to do with the opening of the oscula, the use of the same water over and over again ought soon to bring on a condition that would no longer cause the oscula to open. But sponges placed in the current of the middle aquarium remained with their oscula open for hours in seawater that had been used many times over. Moreover the oscula closed quickly when the current was cut off and reopened soon after it was started again. I therefore believe that the mechanical stimulation of a current of water is an effective means of opening or keeping open the oscula Stylotella.

These first experiments were made on whole colonies of Stylotella and only the general condition of their oscula was recorded. I next turned to the individual sponges, the so-called fingers, to ascertain what parts of the finger must be exposed to the current to induce an opening of the osculum or the reverse. To test this question I placed a colony of Stylotella in a strong current of seawater and, when the oscula were well opened, I lowered a glass tube over a vertical finger so that the tube protected the whole length of the finger from the laterally impinging current but was at no place in contact with the finger. The water in this tube on examination was found to be for the most part quiet; its condition, however, did not interfere with the slight currents produced by the sponge itself. Although the osculum of the finger under examination was fully

open when the tube was lowered over the finger, it closed in seven minutes after the tube was in position and remained so for a quarter of an hour. I now inserted a small tube into the upper end of the large tube and ran a gentle current of seawater down into the end of the large tube where the finger was situated. Thus the sponge was again in a current of seawater and in fourteen minutes its osculum was fully open. On cutting off this current, the osculum closed in six minutes. From these experiments it is quite evident that when no current of seawater impinges on a finger, its osculum closes and when such currents do strike the finger the osculum opens. It was noteworthy that during the time of these experiments the oscula in the immediate neighborhood of the one tested showed no changes in reference to those observed in the individual within the tube, but they remained for the most part persistently open in the general current of seawater.

The next question that naturally suggested itself was how much of a finger must be exposed to a current of seawater to induce the opening of its osculum. To test this, I placed the glass tube over the distal half of a finger leaving the proximal half exposed to the general current. I found, however, that the current eddied up into the tube and thus impinged on a part of the sponge supposed to be protected from it. To check this I inserted a small ring of cotton-wool between the free end of the tube and the sponge. Under these conditions the osculum closed in eight minutes even though the lower half of its finger was in a strong current of seawater. This form of experiment was repeated with only the distal fourth of the finger protected from the current, and again the osculum closed in seven minutes. Thus it is only necessary to have quiet water around the outermost fourth of a finger to cause its osculum to close, and a strong current on the proximal three-fourths of the finger will not induce the osculum to open.

I next reversed these experiments and attempted to ascertain how much of the distal tip of a finger must be exposed to a current to induce the opening of its osculum. In making these trials, a piece of light-weight brass-tubing was cut to such a length that when it was slipped down over a vertical finger of the sponge, it

covered the finger all but the tip. The space between the oscular tip and the tube was filled with cotton-wool and the whole allowed to stand in quiet seawater. After the osculum had been closed for about a quarter of an hour, a gentle current was started across the end of the tube so that it impinged on only the oscular membrane. In three minutes the osculum showed signs of opening and in eight minutes it was fully open. This form of experiment was many times repeated with essentially similar results. It is therefore necessary for the current to impinge on only the oscular tip of the finger in order that the osculum shall open. The closing of the osculum in quiet water and its opening in a current of water are then both very local reactions and cannot be induced from points on the finger a quarter of its length (about half a centimeter) from the osculum.

If the oscula Stylotella close simply because the water in their immediate vicinity ceases to move and not in consequence of the accumulation of waste products or lack of oxygen, they probably close in the air on a falling tide because of the same mechanical conditions. If in the laboratory an inverted test-tube full of air is lowered over a finger whose osculum is open till the oscular membrane just comes in contact with the air, the osculum closes in about three minutes. The same result can be obtained when the test-tube contains washed hydrogen in place of air. Hence this reaction is not due to the oxygen of the air, but is very probably induced by a purely mechanical condition of quiescence into which the tip of the sponge finger passes in going from the water into the gas.

If an osculum opens to the mechanical stimulation of a current and closes in its absence, it is reasonable to suppose that it might respond to the stimulation produced by touching it with a bristle or stroking it with a fine brush, but my attempts in these directions were not conclusive. Touching or stroking an oscular membrane inside or outside when the osculum was open and in a current of seawater never resulted, as might have been expected, in a contraction of the osculum. Similar attempts on the outside of a closed osculum in quiet seawater occasionally resulted in a partial opening of the aperture, but these occurrences were so irregular

and at such lengthy periods after the application of the stimulus that no reliance could be placed on them.

So far as my observations on mechanical stimulation go and they are full only in reference to currents, it is quite clear that an osculum closes quickly (in from about three to eight minutes) in quiet seawater or air, and opens more slowly (in from about seven to fourteen minutes) in a current of seawater. The fact that the oscular closure is quick and its opening relatively slow supports the view that I have already advocated from the standpoint of the structure of these parts, namely, that the sphincters are myocytic and work against the general elasticity of the surrounding tissues. Hence closure might be expected to be rapid and expansion relatively slow.

b. Injury

In making preparations of Stylotella for physiological tests it became quite apparent that the closing of the osculum was a common accompaniment of cutting the sponge. If a finger of Stylotella is cut off about a centimeter from the osculum, that aperture even in a current of seawater is likely to close within a short time and to remain closed for an hour or more. The occurrence of an oscular closure is much less likely, if the finger is cut off at two centimeters from the osculum than at one centimeter. If the cut is made at half a centimeter from the osculum that opening closes very quickly and may remain so for as much as a day.

If a finger instead of being cut off, is only cut into one on side, there is less likelihood of contraction than in the preceding cases. A finger cut into on one side at one centimeter from the osculum retained an open osculum, and the same was true when the cut was half a centimeter from the osculum. But when a cut was made three millimeters from the osculum, this aperture closed in nine minutes and remained so over a quarter of an hour.

If a pin is stuck into a finger of Stylotella, its influence on the osculum depends on the distance it is from that aperture. At one and a half centimeters no certain response was observed, but at half a centimeter the osculum closed in about ten minutes and remained so several hours, though it eventually opened. This

observation is in accord with what Merejkowsky ('78, p. 13) found to be true of Rinalda; if the oscular edge of the sponge is struck several times with a needle, the osculum quickly contracts and remains so several minutes, after which it more slowly opens. The same is said by Merejkowsky (p. 14) to occur in Suberites.

As might be inferred from the statements already made, the injuries done to one finger of Stylotella have no influence on the condition of the oscula of neighboring fingers, nor do injuries inflicted on the common flesh of the colony between fingers influence the oscula of these fingers.

The nature of the stimulus produced by cutting the flesh of a sponge seems to be rather mechanical than otherwise. Such an injury besides mechanically disrupting tissues does little more than liberate juices from the substance of the sponge. These juices, however, when collected and discharged artificially and with great freedom in a normal sponge with open oscula, do not cause the oscula to close. I am therefore led to believe that the closing of the osculum on injury to an adjacent part of the sponge is due to the mechanical disrupting of tissues rather than to the effects of the juices that are liberated. If, however, the stimulus from the injury is chiefly mechanical, it results in a very different form of response from that due to currents, for the latter cause an opening of the osculum while the former induce its closure.

c. Chemical Stimulation

Since the oscular sphincter of Stylotella is made up of tissue that has a striking resemblance to smooth muscle, I tried the effects of a number of drugs on this sphincter to ascertain whether or not they influenced this organ as they did the smooth muscle of the higher animals. The drugs used were ether, chloroform, strychnine, cocaine, and atropin dissolved in seawater. This water was then used in the circulating apparatus already described (p. 12), so that sponges could be exposed to it in currents. I also tested in the same apparatus the effects of diluted seawater, of freshwater, and of seawater deprived of its oxygen by boiling.

When a sponge whose oscula were open in a current of pure seawater suddenly had this changed for a current of seawater con-

taining a half per cent of ether, the oscula closed in from three to three and a half minutes. When in place of pure seawater, seawater containing a half per cent chloroform was used the oscula closed in a minute and a half to two minutes. The sponges treated with ether-water reopened their oscula in about two hours; those that had been subjected to chloroform-water did not reopen in less than four hours and some never recovered. Since both ether and chloroform-water induce a closure of the oscula, even when they are applied to the sponge in the form of a current, I regard these drugs as vigorous stimulants and of the two, chloroform is the more effective and, as in so many other cases, the more harmful.

When a current of seawater containing one part of strychnine to fifteen thousand parts of seawater was substituted for a current of pure seawater, the sponges closed their oscula in from eight to twelve minutes. Thus strychnine must be regarded as a stimulant to contraction.

Sponges whose oscula had remained open for some time in a current of seawater, were subjected to a current containing one part in a thousand of cocaine, whereupon their oscula closed in from seven to ten minutes. All such sponges reopened their oscula after having been in a current of pure seawater for about half an hour. Since the sponges closed even in a current, cocaine at the strength used must be regarded as a vigorous stimulant to closure.

In a solution of one part of cocaine in ten thousand parts of seawater, the oscula remained open as in pure seawater, but, as the following observations show, this drug was not without its effect. A particular osculum was found on several trials in pure seawater to close in from four to five minutes after the current had ceased. On subjecting this osculum for some fifteen minutes to a current of cocaine in seawater, one to ten thousand, it was found that on the cessation of the current the osculum closed in from eight to nine minutes. After an hour in a current of pure seawater the rate of four to five minutes was reëstablished. A weak solution of cocaine, then, inhibits slightly the closure of the osculum.

A cocaine solution of one part in fifty thousand of seawater

could not be distinguished in its action on the osculum from pure seawater.

A solution of one part of atropin to one thousand parts of seawater checked the rapidity of oscular contraction much as the stronger of the two effective solutions of cocaine did, and a solution of one part of atropin in ten thousand parts of seawater could not be distinguished from pure seawater.

Although the observations on the actions of these various drugs as given in the preceding paragraphs are insufficient to admit of any detailed analysis, the results are in agreement with what is known of the action of these materials on smooth muscle. To this type of muscular tissue chloroform is more destructive than ether, strychnine renders it especially contractile, and cocaine and atropin inhibit this property somewhat (Grötzner, '04, p. 65). This evidence, therefore, supports the view that the sphincter myocytes of sponges are in the nature of primitive smooth muscle fibers.

The effects of dilute seawater and of freshwater itself on the oscular mechanism were tried in the circulating apparatus. If a sponge whose oscula have been open in a current of seawater for over an hour is flooded with a current of water composed of one-fourth fresh water and three-fourths sea-water, the majority of the oscula contract somewhat in twenty minutes, after which they remain partly open. In a mixture of half freshwater and half seawater the oscula contract but do not close completely. In three-fourths freshwater and one-fourth seawater, the oscula contract in about seven minutes but do not close. In pure freshwater, they remain expanded as though dead, but even after having been twenty-four minutes in fresh water, such sponges will revive in running seawater, though their oscular collars are seriously damaged and are regenerated only after several days. As Stylotella inhabits the shallow waters near the shore, it must often be subjected after heavy rains to the effects of diluted seawater, but, as the observations recorded above indicate, it would not be seriously damaged by these changes and would probably protect itself against them by oscular constriction.

To prepare seawater free from oxygen a large volume was

boiled vigorously for some time to discharge the contained gas and after this had been accomplished the water was set aside in a tightly stoppered vessel to cool. Sponges in a current of normal seawater and with open oscula were suddenly subjected to a current of seawater thus deoxygenated drawn with as little exposure to the atmosphere as possible from the storage vessel. Their oscula closed in from ten to twelve minutes. On returning them to a current of ordinary seawater, they reopened their oscula in from fifteen to twenty-five minutes. Lack of oxygen will therefore cause the oscula to close.

d. Heat and Cold

The seawater in which Stylotella was found living in the neighborhood of the laboratory in June and July had a temperature of about 25° to 28° C. In a current of this water the oscula of Stylotella will often remain open many hours together. If the temperature of the current was changed to about 35° C., the oscula often constricted slightly, and the same was true at 40° C. At 45° C. the oscula in five or six minutes went into a state of flabby contraction, and if this temperature was maintained for a considerable time much of the sponge died. At temperatures lower than the normal, from 25° to 9° C., the oscula remained open and outward currents could be demonstrated. Thus low temperatures were apparently without effect on the oscula and high temperatures called forth a partial contraction.

e. Light

Sudden changes from the most intense sunlight to the most complete darkness were not followed by any observable movement of the oscula in Stylotella, which in this respect follows the general statement made by Minchin ('00, p. 89), that adult sponges are not sensitive to light.

B. Movements of the Dermal Pores or Ostia

The movements of the dermal pores or ostia in Stylotella were not so easily demonstrated as those of the oscula were. The small

size of the ostia makes a direct determination of their condition almost impossible and consequently the presence or absence of a current of water through them was taken as an indication of their state. The demonstration of this current has been accomplished from the earliest times (Carter, '56, Lieberkühn, '56, Bowerbank, '58) by the addition to the water of some such substances as carmine, starch, or indigo, whose particles could then be followed as they were carried in the moving water. Latterly this method has been severely criticised by von Lendenfeld ('89, p. 592), who claims that even these small suspended particles mechanically stimulate the sponge and cause it to close its ostia. Von Lendenfeld has used milk as an indicator and has found no objection to it. With Stylotella it is easy to demonstrate the ostial currents with carmine, etc., and so far as I could discern this material could be used without causing partial closure of these apertures. In fact I must agree with Bidder ('96, p. 32) that the carmine particles seemed to have no effect whatever on the ostia, but were swept into the interior of the sponge with great freedom for hours at a time. It must, however, be confessed that not only carmine but even milk is an unnatural substance for a sponge and as Stylotella lives in water that ordinarily contains much fine suspended material, I found it necessary only to watch this substance to gain all the information that was needed as to the direction of ostial currents, their strength, etc.

In testing the ostia I usually pinned a finger of sponge under the microscope in a small glass aquarium so arranged that a continuous current of seawater could be kept running through it, and by watching the suspended particles along the sides of such a preparation under a magnification of about ninety diameters, it was comparatively easy to ascertain whether the ostial currents were running or not. As a rule the objective of the microscope was used as an immersion lens and plunged under the surface of the seawater. In making these observations it was, however, necessary for the time being to stop the current of seawater that was running through the small reservoir, otherwise the movement of the suspended particles over the surface of the sponge was so rapid that it was impossible to tell whether they entered an ostium or glided

by it. When this current was shut off the osculum often closed and under such circumstances, as might have been expected, the ostial currents ceased. To be certain that the cessation of these currents was due to the closure of the outlet, I cut off a closed osculum and found that the ostial current almost immediately began again. Moreover when I ligated the cut oscular end of a finger on which ostial currents could be easily seen these currents ceased at once and on the removal of the ligature the currents recommenced. From these observations it is quite clear that the osculum controls in a purely mechanical way the current within the sponge. When the osculum is open this current may run; when it is closed the current ceases even though the ostia are open and the choanocytes continue to beat. In view of these facts I regularly removed the oscular ends from fingers of Stylotella on which I wished to test the ostia.

Although the presence of an ostial current is conclusive evidence of the openness of the ostia, its absence is not proof that the ostia are closed even supposing that the oscular end is cut off, for it is conceivable that the choanocytes may cease to beat, in which case the cessation of the current would be misleading as to the condition of the ostia. For some time I was puzzled as to a means of meeting this difficulty, but a simple method finally suggested itself and was adopted. If the oscular end of a finger of Stylotella is cut off at some distance from the osculum, the cut face includes not only the gastral cavity and some of the flagellated chambers, but also the sub-dermal cavities. An examination of the currents from such a cut end will show a large, slow, central current emerging from the gastral cavity and a considerable number of smaller more rapid currents entering the surrounding sub-dermal cavities. These cavities form a set of intercommunicating spaces over the whole surface of the sponge, and the currents that set into them at the cut end depend purely upon the action of the choanocytes. If, now, no inward currents can be detected at the ostia but currents can still be seen to enter the sub-dermal cavities at their cut ends, it is clear that the absence of lateral currents is due to the closure of the ostia and not to the cessation of the choanacytes. In this way, then, I used the presence of ostial currents to indicat

that the ostia were open and their absence, when coupled with the presence of sub-dermal currents, to indicate that these apertures were closed. Most of the tests that were carried out on the oscula were repeated on the ostia and the results will be stated briefly in the following paragraphs.

a. Mechanical Stimulation

A finger of Stylotella from which the oscular end has been cut off may be kept a long time in running seawater in apparently normal condition. When the current of seawater is temporarily shut off, small suspended particles can be seen to drift slowly up to the surface of the finger and disappear by suddenly darting into the ostia. In this way the ostia can be demonstrated to be open. Many particles are too large to enter these apertures and they will accumulate on the surface of the animal in quiet water. When, however, the general current is set going again, it sweeps these larger particles away and leaves the surface of the sponge relatively clean. If after the ostia have been demonstrated to remain open for some time in running seawater, this current is permanently shut off so as to leave the sponge in quiet water, the ostia may con tinue open for many hours during which the surface of the sponge often becomes deeply buried under an accumulation of particles most of which are too large to enter the ostia. In no case has a cessation of the seawater current been followed by a closure of the ostia as with the oscula. The ostia, then, differ from the oscula in that they remain open in both quiet and circulating seawater.

Prepared fingers of Stylotella in which strong sub-dermal and oscular currents could be seen but no ostial currents were persent, were kept in some instances in running seawater, in others in quiet seawater without, however, yielding any evidence to show that either state of the seawater caused the opening of the ostia. Flowing seawater and quiet seawater both seem to have no effect on the opening or closing of the ostia in Stylotella.

Even when Stylotella is covered with a deep layer of silt, its ostia can often be demonstrated to be open. Under ordinary circumstances, however, it is not usual to find this sponge thus

covered. Its natural habitat is in a current of seawater and though this water may often be heavily loaded with suspended matter, its current seems to be sufficient, as already noted, to remove many particles which, from their size, accumulate on the surface of the sponge. But this is not the only means for the removal of these larger particles. A close inspection of the outer surface of Stylotella will show that it is regularly inhabited by several animals; chief among these are young ophiurans, caprellas, and a species of copepod. All these animals, and especially the copepods, keep up an incessant movement over the surface and loosen and dislodge much of the accumulated drift. The copepods and probably the other forms find much to feed on in this omnium-gatherum, and their relation to the Stylotella seems to be of a symbiotic character. These organisms together with sea currents are responsible for the generally clear character of the surface of the sponge. But even when this sediment is abundantly present on Stylotella its ostia remain open. I have also failed to find any evidence in favor of the view that when the ostia are closed the accumulation of silt on the surface of the sponge will cause them to open.

Exposure to air likewise seems to have no effect on the ostia. A finger of Stylotella on which the ostia were freely open was exposed to air for about a quarter of an hour. Upon reimmersing it in seawater the ostial current could be seen at once. It was again put in the air, this time for three-quarters of an hour, whereupon it was reimmersed and the ostia again gave evidence of being freely open though the finger as a whole had the shriveled appearance characteristic of sponges that have been exposed sometime to the air.

Stroking the surface of Stylotella seems neither to bring about a closure nor an opening of the ostia. In this respect they are as irresponsive as the oscula.

So far as mechanical stimulation is concerned, the ostia are very unlike the oscula. The oscula are responsive to water currents and their absence and the mechanical effects of exposure to the air; the ostia are uninfluenced by any of these changes and are apparently also undisturbed by sediment.

b. Injury

The great majority of fingers cut from Stylotella, if put directly under the microscope, show no ostial currents. As a rule these currents begin to appear in from ten to fifteen minutes after the finger has been cut off. When a finger that has established its ostial currents is cut in two, these currents often cease in the two parts though sub-dermal and oscular currents can be easily demonstrated. After a quarter of an hour the ostial currents can usually be seen again in such pieces. Not all specimens show these conditions, but they are of common enough occurrence to justify the conclusion that a considerable incision in Stylotella produces in its neighborhood a temporary closure of the ostia. In this respect the ostia resemble the oscula.

c. Chemical Stimulation

To seawater containing ether ($\frac{1}{2}$ per cent) or chloroform ($\frac{1}{2}$ per cent) the ostia closed even more quickly than the oscula did, and on fingers whose ostia were closed, the presence of ether or chloroform in the surrounding water did not induce these apertures to open. Strychnine, one part in fifteen thousand of seawater, was followed by a gradual closing of the ostia, a condition already observed by von Lendenfeld ('89, p. 608) in other sponges. To one part of cocaine in a thousand parts of seawater open ostia closed in about ten minutes and closed ostia remained closed. To one part of cocaine in ten-thousand parts of seawater the ostia remained open or if closed in the beginning, they open in about eight minutes. Of this drug von Lendenfeld ('89, p. 640) states that the stronger solutions cause a contraction of the ostia, which is true, and that the weaker solutions leave these apertures unchanged, which is probably not wholly correct, for they inhibit to some extent the contractibility of the sphincters. To atropin, one part in a thousand of seawater, the open ostia remained open and the closed ones opened in about nine minutes. Thus atropin probably also inhibits the action of the sphincter. As the actions of these various drugs on the ostial sphincters is very similar to their action on smooth muscle, it is probable that the ostial myo-

cytes, like those of the osculum, are cells not inappropriately described as primitive smooth muscle-fibers.

In water composed of three-quarters seawater to one-quarter freshwater, the ostia remained open, and a strong ostial current could be seen at the end of twenty minutes. When the ostia were closed the effect of this mixture was to induce an opening of the ostia in about a quarter of an hour, after which a strong ostial current continued to flow. To mixtures of half seawater and half freshwater oscular, and sub-dermal currents as well as ostial currents ceased in about ten to twelve minutes, showing that though the ostia probably remained open, the currents ceased because of the collapse of the choanocytes. Closed ostia in this mixture opened slightly and then all currents ceased in from nine to ten minutes. In fresh water all currents ceased immediately. These mixtures of seawater and freshwater so far as their effects can be seen, influenced the ostia much as they did the oscula, in that they induce a partial but imperfect contraction.

In seawater rendered free from oxygen by boiling and subsequently cooled to 28° C., the ostia remained open or, if closed, they opened in from seven to ten minutes. This reaction was precisely the reverse of that of the osculum and to make close comparisons of the two I prepared several fingers of Stylotella by cutting them off rather short at the proximal ends thus permanently opening the gastral cavity and by leaving the oscular end intact. These preparations were placed one after another under the microscope in pure running seawater and after the oscula were freely open the current of pure seawater was changed for one of deoxygenated seawater. Under these conditions all the oscula closed in about ten minutes, if not completely at least nearly so, and the ostia remained open, their currents now discharging chiefly through the cut end of the gastral cavity. Deoxygenated water, then, is a means of closing the oscula and opening or leaving open the ostia.

To seawater containing juice expressed from an oyster, either fresh or foul, the open ostia remained open and their currents seemed at times to increase. I was never able to demonstrate with certainty that to these materials the closed ostia would

open, though occasionally they seemed to. Like the oscula, the ostia were indifferent to seawater containing juice from the body of Stylotella itself.

d. Heat and Cold

Prepared fingers of Stylotella in which the ostial currents were running vigorously continued to exhibit these currents after the temperature of the seawater had been changed from 28° C. to 36° C., and fingers in which the ostia were closed at 28° C. opened them after the sponge had been a few minutes in water at 35° C. At 40° C. all currents, sub-dermal and oscular as well as ostial, became rapidly feeble and then stopped, and at 45° C. these currents ceased abruptly as though the heat had caused the choanocytes to stop beating. This view is supported by the fact that few fingers of Stylotella ever recovered after having been subjected to seawater at 45° C. for any length of time. Cold water at 9.5° C. caused all currents to run more slowly, but did not bring about a closure of the ostia. In fingers in which the ostia were closed these organs did not open after having been a quarter of an hour in seawater at 9° C. In these specimens the subdermal and oscular currents became sluggish on reducing the temperature of the water, hence the effect of the low temperature was probably chiefly on the choanocytes.

e. Light

As in the case of the osculum, I have observed no effect from intense sunlight or shadow on the opening or closing of the ostia.

C. Movements of the Body as a Whole

Aristotle in the fourteenth chapter of his fifth book on the history of animals makes the interesting statement that the sponge is supposed to possess sensation because it contracts if it perceives any movement to tear it up and it does the same when the winds and waves are so violent that they might loosen it from its attachment. He further adds in his characteristic way that the natives of Torona dispute this. The idea that the common

flesh of the sponge is contractile is not without modern support. Merejkowsky ('78, p. 14) states that if Suberites is so placed that it is partly out of water, it will curve the body until it is under water as much as possible, and if the body is then covered with water, it will return to its former position.

It must be evident, from what has already been stated, that much of the common flesh of Stylotella is contractile. As already noted, specimens our of water quickly assume a shriveled and rugose appearance as though the flesh had contracted on a resistant skeleton, a condition which it also quickly assumes in quiet seawater. Moreover, if a sponge is placed partly in running seawater and partly in the air, the portion in the seawater remains smooth and that in the air becomes rugose. Specimens made rugose either in the air or in quiet water soon recover their smooth appearance on being placed in running water. Air or quiet water may then cause a contraction of the common flesh of Stylotella, a condition counteracted by running water.

The contraction of the common flesh can also be seen well around some of the larger cavities, such as the gastral cavity. If a long finger of Stylotella whose two ends have been cut off and whose gastral cavity extends along one of its sides is placed in quiet seawater, the gastral cavity is soon indicated by an external groove due to the apparent collapse of its wall. This groove, however, is caused not by collapse, but by the contraction of the common flesh which as partial partitions or even travecula is abundant about the sides of the gastral cavity. On returning the finger to running water the flesh relaxes and the groove mostly disappears.

Although the common flesh of Stylotella is unquestionably contractile, I have never observed that the body of this sponge as a whole moves in consequence of this contractility. Thus in no instance have I seen a partly immersed finger of Stylotella bend farther into the water, though I have let fingers stand in a position favorable for this for over a day. Nor have I ever observed fingers to turn in conformity to the direction of the current. Thus some fingers of Stylotella are not directed straight upward, but have their tips turned to one side or the other, so that the oscula open laterally. A number of these were set, some with oscula fac-

ing the current, some with these openings away from the current, and others sidewise to the current. After three days none of these had materially changed their directions, thus giving no evidence of a general movement of the body.

I also attempted to get evidence of the general movement of the body through geotropic responses. Stylotella ordinarily grows with its fingers and oscula directed upwards, as though it was negatively geotropic. A large colony was, therefore, kept inverted in an aquarium of circulating seawater for about a week on the assumption that the fingers might turn from this unusual position, but at the end of this period there was no apparent change of position. This observation, however, does not prove that Stylotella is not geotropic. Slight evidence of geotropism is to be found in its method of regenerating oscula. When a moderately long finger of Stylotella is cut off and the whole of its oscular end, removed, the cylindrical body thus resulting will under favorable conditions form a new osculum. Whether this regeneration will take place at the end nearer or farther from the former osculum seems to depend chiefly on the position of the piece of sponge in reference to gravity. If the end that was nearer the former osculum is uppermost, it always regenerates the new osculum; if it is down, the opposite end very generally regenerates the new organ. Thus in the regeneration of the osculum Stylotella shows some slight geotropic activity, and while it must be admitted that the common flesh of this sponge is contractile, this contractility does not seem to result in movements of the body as a whole such as might be looked for in geotropic and other like responses. It is possible that in this sponge the skeleton, which is well developed, is too resistant to allow the body as a whole to be bent, and that, therefore, the contractility of the common flesh can make itself manifest only in the local ways already mentioned.

D. Currents

The currents of sponges, which were supposed by many older naturalists to reverse in their direction from time to time and to depend upon a systole and diastole of the body of the sponge, have

been generally acknowledged since the time of Grant ('25, '26, '27) to be uniform in their direction and to depend upon the action of cilia-like organs. Some years ago Miklucho-Maclay ('68) and Haeckel ('72) maintained that a reversal of the current could occur, but more recent observers have not confirmed this statement. In the thousands of living individuals of Stylotella that I have examined I have never seen an exception to the rule that water enters the ostia and sub-dermal cavities, when open, and makes its exit through the osculum. Moreover I have never found a living specimen of Stylotella in which currents could not be demonstrated. Even in those in which the ostia and oscula were closed and no external evidence of currents could be seen, the cutting off of the oscular end and the consequent exposure of the gastral and sub-dermal cavities always was followed by the appearance of characteristic currents. It seems to me probable that under nurmal conditions the choanocytes beat incessantly in Stylotella. The currents produced by them would then be controlled by the opening and closing of the ostia and the oscula. It must be borne in mind, however, that a continuous current does not necessarily mean that all flagellated chambers are continuously at work. Some may cease from time to time without causing the general current to cease. All that the presence of a continuous current really proves is that all flagellated chambers are not inactive at once. It would not be surprising to me, however, to find, if evidence could be obtained, that the action of the choanocytes is uninterrupted. The fact that a current could always be demonstrated in all fingers of Stylotella by cutting off the oscular ends leads to the conclusion that, aside from the ostia and the oscula, there is no other complete check on the current such as prosopylic or apopylic sphincters, etc.

If the ostial and oscular sphincters are the organs of control for the currents in a sponge, they must be strong enough to resist the pressure produced by these currents, and when these apertures are closed the tissues of the sponge must also withstand a certain strain produced by the working of the choanocytes. Doubt has been expressed by some writers as to the ability of the body of a sponge to meet these mechanical requirements, but as no one, so

far as I am aware, has ever attempted to measure the pressures involved, it seems useless to urge such objections till actual measurement has been accomplished. It is comparatively easy to determine the pressure produced by the activity of the choanocytes of such a sponge as Stylotella. To make this measurement the following simple device was employed. A glass tube of about five millimeters bore was drawn out at one end to a diameter of about one millimeter, and fixed vertically at such a height that its pointed end was well under the surface of the seawater in an aquarium. The water of course rose in this tube to the level of that in the aquarium. A long finger of Stylotella in which the currents were running well was ligated at its cut end so that no water could escape from this end, and the osculum was fitted over the small end of the glass tube and firmly tied there. The sponge continued to pump water in through the ostia, and this water naturally rose in the glass tube until the pressure of the column of water in the tube just neutralized the strength of current produced by the choanocytes. This position having been read on a scale attached to the glass tube, the finger of the sponge was then carefully cut off from the tube, whereupon the water fell in the tube almost to the level of that in the aquarium, the difference being due to the capillarity of the tube. This new level was then read and the difference between the two levels was taken to represent the pressure exerted by the current produced by the sponge. Ten such trials were made and in all cases the readings fell between 3.5 mm. and 4 mm. The current produced by Stylotella, then, has a maximum pressure equivalent to a column of water between 3.5 and 4 mm. high. This slight pressure is what must be resisted by the tissues, and particularly the sphincters of Stylotella when in a closed condition of the sponge the choanocytes continue to beat. To ascertain what the resistance of the sphincters was, I subjected them to a simple test. A finger of Stylotella in which the ostia were closed was tied as before to the small end of a glass tube which was bent in the form of a siphon and was so placed that the end carrying the sponge was in one vessel of water and the other end, quite free, was in another vessel of water. The water in these two vessels was kept at the

same level. After the whole apparatus was set up the water in which the sponge rested was deeply colored with methyl green. The vessel with uncolored water was then lowered till the difference in level between the water contained in it and in the other vessel was sufficient to break through the ostial openings, a state of affairs that could be recognized by the passage of the deep bluish green water up one arm of the siphon. The difference in level was then measured and in the eight trials that were made it was found to be between ten and fifteen millimeters. Thus the actual amount of resistance in the closed ostia is much more than is necessary to hold in check a current whose maximum suction is represented by a pressure of not over four millimeters of water. I also attempted to get the resistance of a closed osculum. Oscular tips were tied to the small end of the siphon tube, which in this instance was made to carry colored water, and by raising the reservoir on the colored-water side a pressure was sought at which the osculum would open and discharge colored water. But my experiments failed mostly because of leakage, probably through the ostia near the osculum. They went far enough, however, to assure me that the resistance of the osculum was higher than that of the ostia. From these observations it is quite evident that the currents produced by the choanocytes of Stylotella are of such a strength that they can be readily held in check by the ostia and the oscula, and that there is no mechanical ground for suspecting that these currents could in any physical way endanger even the delicate structure of the sponge.

The experiments with various stimuli had in many cases little or no observable influence on the currents produced by the choanocytes. The mechanical stimulation of the exterior of the sponge had no effect on the current. In ether- and chloroform-water all currents ceased, as might be expected from the well-known inhibitory action of these drugs on cilia, etc. Strychnine apparently increased the vigor of the current, whereas cocaine and atropin seemed to have no effect upon it. Dilute seawater and fresh water brought the current quickly to a standstill. Lack of oxygen first accelerated and then retarded it. Cold caused it to become slow, and excessive heat brought it to a standstill.

Light, as might have been expected, did not alter it. So far as these various stimuli change the current at all, they do so as one would expect of them supposing that they acted directly on the choanocytes and not through any intermediate structure. Nevertheless it cannot be said from the evidence presented that the complete cessation of the current, as for instance in fresh water, may not be due to the contraction of sphincters other than the ostia and oscula rather than to direct action of the choanocytes.

E. Coördination of Reactions

A comparison of the reactions of the oscula, ostia, and choanocytes of Stylotella, as described in the preceding sections, can best be made through a summary such as is contained in Table 2.

The most marked feature brought out in Table 2 is the very striking independence of the several reactive organs. Thus the activity of the choanocytes, as indicated by the current they produce, is apparently quite independent of that of the oscula or the ostia and the only stimuli that have any effect on these cilia-like organs are such as would be expected to influence them directly. The oscula and ostia both possess sphincters that from a histological standpoint are much alike in that they probably are a primitive form of smooth muscle, a view supported by their reactions to drugs, and yet they are influenced in totally different ways by several stimuli, especially of a mechanical kind. This is so striking that under natural conditions one of these sets of apertures may be found open when the other is closed. The contraction of the common flesh is also quite independent of the ostia, for, though these apertures are in a measure imbedded in this flesh, the latter may be contracted when the ostia are open. The contraction of the flesh and the closure of the oscula always take place together when the sponge is in quiet water or exposed to the air, but that this is probably a coincidence rather than evidence of a real physiological interdependence is seen from the fact that the oscula close in ether- and chloroform-water, though the common flesh does not contract in these media. Thus the various motor elements in Stylotella seem to be as independent of one another as the several parts of a single animal can well be.

TABLE 2

Summary of the Reactions of the Oscula, Ostia, and Choanocytes of Stylotella to Various Stimuli

STIMULI	REACTION BY		
	Osculum	Ostium	Choanocyte
Mechanical Stimuli			
Seawater currents	Opens and remains open	No reaction	No reaction
Quiet seawater	Closes and remains closed	No reaction	No reaction
Brushing	No reaction?	No reaction	No reaction
Silt		No reaction	No reaction
Exposure to air	Closes and remains closed	No reaction	No reaction
Injury	Closes	Closes	No reaction
Chemical Stimuli			
0.5 per cent ether	Closes and remains closed	Closes quickly and remains closed	Currents soon cease
0.5 per cent chloroform	Closes and remains closed	Closes quickly and remains closed	Currents soon cease
1/15,000 strychnine	Closes and remains closed	Gradually closes	Currents strong
1/1,000 cocaine	Closes and remains closed	Closes and remains closed	No reaction
1/10,000 cocaine	Remains open; closure inhibited	Remains open or if closed, soon opens	No reaction
1/50,000 cocaine	Remains open		No reaction
1/1,000 atropine	Remains open; closure inhibited	Remains open or if closed, soon opens	No reaction
1/10,000 atropine	Remains open		No reaction
3/4 seawater + 1/4 freshwater	Closes slightly, then reopens	Remains open, or if closed, opens	No reaction
1/2 seawater + 1/2 freshwater	Contracts but does not close	Probably remains open; if closed, opens slighty	Currents soon cease
1/4 seawater + 3/4 freshwater	Contracts but does not close		
Freshwater	Remains open but inactive	?	Currents cease
Deoxygenated water	Closes	Opens and remains open.	Currents strong, then cease
Thermal Stimuli			
9° to 10° C	No reaction	No reaction	Currents become slow
25° to 28° C	Normal	Normal	Normal
35° C	Slight constriction	Remains open or if closed opens	Normal
40° C	Slight constriction	?	Currents cease
45° C	Flabby contraction	?	Currents cease
Light	No reaction	No reaction	No reaction

This organic independence in Stylotella also appears in the almost complete absence of transmission from part to part. The opening and closing of an osculum on one finger in accordance with the condition of the surrounding water has no influence on adjacent oscula even though they be only a centimeter or so distant. Extensive wounds, which can be made with much local precision, influence oscula or ostia only within a very close range. Transmission at best cannot be over a much greater distance than a centimeter or so. Nor is the nature of this transmission at all nerve-like. A cut made about 3 mm. from an osculum was followed by the closure of the osculum only after eleven minutes, though this osculum had previously closed in quiet water in from four to five minutes. The form of reaction resembles that seen in the vertebrate iris, in which in response to a point of light the iris contracts locally, the contraction gradually spreading through the whole organ (Hertel, '07). In the sponge, as in the iris, we are probably dealing with the direct stimulation of smooth muscle, which when locally contracted stimulates by its contraction the adjoining resting muscle and thus a slow form of transmission is accomplished through the muscle substance itself.

These studies of the reactions of Stylotella support the conclusion arrived at from earlier anatomical investigations to the effect that sponges possess nothing that may with propriety be called nervous tissue. Their reactions, which have the general character of great simplicity and independence, are, I believe, entirely due to the direct stimulation of choanocytes or myocytes which are either on exposed surfaces or close to them and which, at least in the case of myocytes, exhibit a form of progressive stimulation that resembles sluggish transmission. Sponges are metazoans possessing muscular but not nervous tissue.

5. ORIGIN OF THE NERVOUS SYSTEM

In seeking evidence on the origin of the nervous system, investigators have naturally turned to primitive metazoans, and the cœlenterates have afforded the principal material for speculation on this subject. These speculations took their origin in the dis-

covery by Kleinenberg ('72) of the so-called neuromuscular cells in hydra. These cells, which have since been found in great abundance in many other cœlenterates, were believed by Kleinenberg to contain the germ of the nervous and muscular systems of the higher metazoans. According to him the elongated basal process of the neuromuscular cell was the contractile or muscular element, and the cell-body that reached from the exterior to the muscular part was the receptive and transmitting or nervous part. In his opinion this cell became divided into two, one cell to become purely muscular, the other purely nervous, and these two cells, thus derived directly from the primitive neuromuscular cell, were supposed to be the forerunners of the muscular and nervous systems of the higher animals. Kleinenberg thus conceived muscular and nervous organs to have had a common origin and to have undergone a simultaneous differentiation. The neuromuscular-cell theory was favored by Van Beneden ('74), who claimed that in Hydractinia the intermediate condition between a neuromuscular cell and its two derivatives was to be seen, but Bergh ('78) showed this claim to be based on inaccurate observation.

The study of the nervous system and sense organs of marine cœlenterates led Oscar and Richard Hertwig ('78) to the conclusion that the so-called neuromuscular cells were not nervous but merely muscular, and they proposed for these elements the name epithelial muscle-cells. They also pointed out that the nervous system of the cœlenterates consisted of sense-cells and ganglion-cells and they believed that these two kinds of cells together with the epithelial muscle-cells were simultaneously differentiated from among the elements of the cœlenterate epithelium. Thus they did not trace the origin of nervous and muscular tissue to a single cell but to a layer of cells from which the three types just named were supposed to arise by simultaneous differentiation. This view, though slightly modified by such workers as Havet ('01), who declared that what the Hertwigs called ganglion-cells were more strictly speaking motor-cells, has been more or less tacitly accepted by most modern students of the neuromuscular mechanism of cœlenterates (Schaeppi, '04; Wolff, '04; Hadzi, '09; Groselj, '09).

The views of Kleinenberg and the Hertwigs, as this brief survey shows both contain the common element of simultaneous and inter-related differentiation of nervous and muscular elements. As contrasted with this aspect of the question Claus ('78) and Chun ('80) claimed an independent origin for these two types of tissue and that their connection was secondary. The ground for this opinion, at least as maintained by Chun, is chiefly the condition found in vertebrates where in ontogeny it is very probable that nerve and muscle are independently differentiated and secondarily united. Thus this opinion gets its support from highly specialized rather than from primitive metazoans.

The view as to the origin of the nervous system, or better of the neuromuscular mechanism, to which the study of the activities of sponges has led me, is in strong contrast with the opinions that have already been expressed. The fact that sponges have an organized musculature, though they show no evidence of nervous organs, leads me to the conclusion that nerve and muscle have not differentiated simultaneously, but that muscular tissue has preceded nervous tissue in order of evolution. The condition in sponges is absolutely contrary to the statement of Kleinenberg ('72, p. 23) that there are no animals with muscles and without nerves, nor is it consistent with the view of the Hertwigs ('78, p. 165) and their followers that these two kinds of tissue differentiate simultaneously. Muscular tissue unassociated with anything that can reasonably be called nervous tissue certainly occurs in these primitive metazoans, and muscle of this kind directly stimulated, i. e., without the necessary intervention of other cells, is in my opinion the initial stage in the growth of the neuromuscular mechanism. The next step in this process is, I believe, that realized in most cœlenterates, i. e., a muscular mechanism to which has been added certain receptive cells, sense cells, that serve as delicate organs for bringing the muscles into action. This step is the first step in the differentiation of true nervous tissue, though it is the second in the growth of the neuromuscular mechanism as a whole. At this point my view is in strong contrast with that of Claus and Chun who, as already stated, have maintained that nerve and muscle arose independently. This I do

not believe possible, for I agree entirely with Samassa ('92) when he declares that a nervous mechanism without muscles or other effectors is inconceivable. In my opinion nervous tissue has differentiated not independently of muscle, as claimed by Claus and by Chun, but in most intimate relations with it and as a more effective means of bringing it into action than direct stimulation is. Primitive muscles, then, as independent effectors, were centers around which the beginnings of nervous differentiation probably occurred, in that certain peripheral cells came to be specialized as receptors for stimuli and excitors of muscular activity, a condition now realized in cœlenterates.

From this standpoint it must be clear that the histogenesis of primitive nervous tissue involves cells that are in contact with the exterior on the one hand and with muscular tissue on the other. The conditions realize almost perfectly the requirements of the well-known theory of neurogenesis advocated by Hensen ('64), and I, therefore, believe that this theory is a truthful portrayal of primitive neurogenesis. I do not admit, however, that it presents a correct picture of the histogenesis of vertebrate nerves. In this problem the evidence seems to me to be strongly in favor of the initial separateness of nerve and muscle and their secondary union, an operation which in my opinion is a cœnogenetic modification of the primitive process. But whether the axis-cylinders of vertebrate nerve-fibers are outgrowth of neuroblasts or not, is a question that has no direct bearing on the one herein discussed, the differentiation of the primitive nervous system. Such a primitive nervous system, essentially receptive in character, is, however, merely the beginning of that structure which in the higher metazoans is designated as nervous. This primitive nervous system is not in any appropriate sense to be called centralized. Its diffuse character, from an anatomical as well as from a physiological standpoint, is well known, and only after the nervous structures have become concentrated either in the periphera lepithelium, as in some worms, or on separation from this epithelium, as in the higher metazoans, is a condition arrived at which necessitates the formation of true nerves, and allows the establishment of common paths (Sherrington, '06), a condition which

may be appropriately called centralized. When this concentration takes place it usually occurs near the chief group of sense organs and gives rise to what is conventionally called the brain. In nervous differentiation, then, the chief central organ or brain follows, in its early evolution, the lines of sensory differentiation. The differentiation of the complete neuromuscular mechanism as possessed by the higher animals, has occurred, I believe, in three successive steps; first, the formation of independent effectors, tor as seen in the muscles of sponges; secondly, the addition of receptors to such effectors, as seen in what I have elsewhere called the receptor-effector systems of the cœlenterates; and finally, the differentiation near the receptors of adjusters or central organs concerned primarily with easy transmission from receptors to effectors (Parker '09).

6. SUMMARY

1 Stylotella under natural conditions closes its oscula and contracts its flesh when at low tide it is exposed to the air.

2 Its outer surface is perforated by many ostia which lead to large subdermal cavities, these in turn connect through incurrent canals with the flagellated chambers from which excurrent canals pass to the gastral cavity and the osculum.

3 The flesh of Stylotella contains many myocytes, which are arranged as sphincters around the ostia, internal cavities, and osculum. These sphincters apparently work against the general elasticity of the flesh and not against radiating systems of myocytes.

4 The oscula close in quiet seawater, on exposure to air, on injury to neighboring parts, in solutions of ether (0.5 per cent), chloroform (0.5 per cent), strychnine ($\frac{1}{15000}$), cocaine ($\frac{1}{1000}$), and in deoxygenated seawater. They contract but do not close in diluted seawater and at temperatures higher than normal (35° to 45° C.). They remain open in currents of seawater, and their closure is inhibited by solutions of cocaine ($\frac{1}{10000}$) and of atropine ($\frac{1}{1000}$), and in fresh water. They are apparently uninfluenced by low temperatures, by weak solutions of cocaine ($\frac{1}{50000}$) and of atropine ($\frac{1}{100000}$) and by light.

5 The ostia close on injury to neighboring parts, in solutions of ether (0.5 per cent). chloroform (0.5 per cent), strychnine ($\frac{1}{15000}$), and cocaine ($\frac{1}{1000}$). They open in solutions of cocaine ($\frac{1}{10000}$). and of atropine ($\frac{1}{1000}$), in dilute seawater, deoxygenated seawater, and warm seawater (35° C.). They are apparently unaffected by mechanical stimulation, except injury, by low temperature, and by light.

6 The choanocyte currents cease in solutions of ether (0.5 per cent), and of chloroform (0.5 per cent), in diluted seawater and at high temperatures (40° – 45° C.). They become slow at low temperatures (9° – 10° C.), and fast in solutions of strychnine ($\frac{1}{15000}$). In deoxygenated water they first become fast and then cease.

7 The flesh of Stylotella is capable of contraction, but such contractions give the sponge only a shrivelled appearance without changing its general form.

8 The currents in Stylotella are constant in direction and give no evidence of reversal. They are controlled by the ostial and oscular sphincters. They produce a pressure equivalent to 3.5 to 4 millimeters of water. The pressure necessary to break through the closed ostia is 10 to 15 millimeters of water and through the closed oscula somewhat more.

9 The reactive organs of Stylotella, the ostia, the oscula, the flesh, and the choanocytes, are all more or less independent of one another and their action is changed by direct stimulation. In the ostia, oscula, and flesh contraction is accomplished by spindle-shaped cells, the myocytes, which resemble primitive, smooth muscle-fibers.

10 The body of Stylotella is almost without transmission and such transmission as is present is so sluggish in character and so silght in range as to resemble transmission in muscles and not in nerves. It is probable that Stylotella possesses no organs that can reasonably be called nervous.

11 The nervous and muscular systems of metazoans were not differentiated simultaneously (Kleinenberg, O. and R. Hertwig) nor independently (Claus, Chun), but muscles, independent effectors, as represented by the sphincters of sponges, were the first of the neuromuscular organs to appear and these formed centers

around which the first truly nervous organs, receptors, in the form of sense-cells developed giving rise to a condition such as is seen in the cœlenterates today. To this receptor-effector system as seen in modern cœlenterates was added in the higher metazoans the adjuster or central organ, thus completing the essential parts of the neuromuscular mechanism as seen in the higher metazoans.

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THE REACTIONS OF ÆOLOSOMA TO CHEMICAL STIMULI

BY

H. G. KRIBS

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INTRODUCTION

The object of this paper is to consider the movements and reactions of Æolosoma under definite conditions of control, as a contribution leading toward an analysis of the nature of its behavior.

Æolosoma is able to move about freely in the ambient medium. The energy which finds expression in the performance of these

movements, for the most part at least, is set free by the rearrangement of certain chemical aggregates within the animal itself. These internal changes which find expression in external movement are a fundamental part of the animal economy. They tend to restore or maintain a certain physiological equilibrium which is essential to its well being.

On the other hand, the ultimate source of the energy which gives rise to these movements comes to the animal through external media. As the animal moves about it is constantly coming in contact with other supplies of energy which have their play in its environment, and there immediately results a mutual reaction between the animal economy and the external supply of energy or "stimulus" thus encountered. The form of the reaction that may be exhibited upon such a contact depends both upon the morphological and physiological organization of the animal, and also upon the sort of stuff that may house the external energy. If this energy be in the form of food particles, for instance, it may be readily appropriated through the various channels of ingestion and assimilation. If it be such as to disturb the internal processes, other and varied movements may result. The sum total of all the movements exhibited by Æolosoma in a given time, in response to a changing environment, we designate as its behavior.

The problem of behavior from this point of view naturally hinges upon the interpretation of the relations which exist between the external stimulus and the concomitant reaction of the animal.

As an introduction to this subject we will first consider themorphological basis of the behavior of Æolosoma; its movements and reactions in a state of nature; and finally physiological effects produced through physical changes in its environment. We will then investigate the movements and reactions of Æolosoma under the control of definite chemical stimulations associated with certain changes in its environments as suggested by these observations on its natural history.

I wish to express my deep obligation to Prof. H. S. Jennings, who suggested this problem to me, and for his kind assistance in

outlining a general method of procedure; to Profs. E. G. Conkin and J. Percy Moore for many valuable hints and corrections.

NATURAL HISTORY

Only very brief references to the natural history of Æolosoma may be found in the literature. Some of these will be noted in passing. No careful investigation into its behavior has been made.

General Morphology

Æolosoma is the only genus thus far described of the Oligochætan family, Æolosomidæ (Aphanoneura, Vedjovsky). It abounds in fresh water ponds and streams throughout the equatorial and neo-arctic regions A schematic drawing of the anima lappears in Fig. 1. The different species vary in length from about 1 mm.

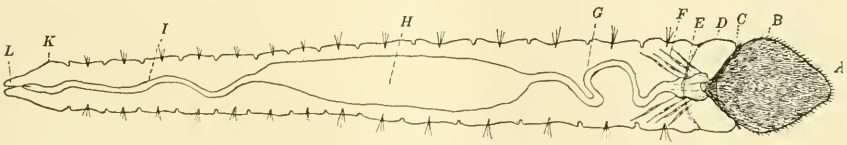

Fig. 1. Ventral View of Æolosoma.

A Sensory hairs	*E* Pharynx	*I* Intestine
B Prostomium	*F* Muscle fibers	*K* Caudal segment
C Buccal cirri	*G* Oesophagus	*L* Papillæ
D Peristomium	*H* Stomach	

(A. quaternarium) to about 10 mm. (A. tenebrarum). The segments, expressed chiefly in epidermal structures, are from 5 to 15 or more in number. The brain, or cerebral ganglia, and the ventral nerve cord, are noteworthy in that they are buried in the epidermis (Vedjovsky '84). Each segment, with the exception of the head segment, and the budding zones, bears four bundles of setæ. The head segment bears a prominent flexible upper lip, or prostomium, which is ciliated on the under side. The prostomium is thin dorsoventrally, and is concave on the under side, making it somewhat spoonshaped. Circling about the tip of the prostomium project little spines or hairs, which are connected

with sensory hairs lying on the inner side of the epidermis (Brace '01). At its base the lateral edges of the prostomium fold into the buccal cavity. The ridge thus formed about the mouth possesses larger cilia than those present on other parts of the prostomium and the beat is correspondingly slower. Surrounding the mouth from the rear and running forward and upward, so as to partially overlap the rear lateral edges of the prostomium, is a a prominent under lip or peristomium. This lip may be so extended that it practically reaches the size of the prostomium, or so far withdrawn that it becomes almost invisible. It is not ciliated The pharynx is a pear shaped organ and very muscular. It is attached to the body wall, caudad, by a series of muscle fibers. The anal segment has two papillæ at its tip, similar in shape and function to the toes of a rotifer. These papillæ seem to have been overlooked in previous observations on the structure of Æolosoma.

There are two layers of muscle fibers, one circular and one longitudinal, lying close to the inner side of the epidermis throughout the body segments. These muscle fibers are capable of enormous contraction and extension. Within the epidermis are numerous gland cells, some of which secrete mucus, while others secrete an oily substance which is scattered in characteristic "globules" throughout the epidermis. There is a thin semitransparent membrane forming the outer layer of the epidermis, and but loosely connected with the other epidermal aggregates.

Æolosoma is hermaphroditic. It also reproduces by the formation of zooids in a way similar to that of Stenostoma. I have never found any specimens that were sexually mature. They have been observed, however, by D'Udekem ('62), Maggi ('65), Stolc ('89), and Nelson ('06).

Movements and Reactions in a State of Nature

The food of Æolosoma consists largely of bacteria, diatoms, unicellular algæ, and the soft mesophyll tissues of decaying leaves. When feeding the pharynx is generally progruded so as to come in contact with the substratum. The peristomium is also con-

siderably extended and spread out on either side. The prostomium is spread out and elevated above the plane of the pharynx and peristomium. The pharynx sucks in the algæ, bacteria, etc., assisted by the beat of the large cilia or cirri of the buccal cavity. When larger pieces of food are to be swallowed the prostomium and peristomium may be used as lips to envelop the substance to be ingested.

As an aid in securing food the small cilia of the peristomium beat incessantly from before backwards, drawing currents of water from in front of the animal and washing them against the cirri of the buccal cavity. By testing these currents with fine india ink granules, it was noted that, in the case of a large A. tenebrarum, the currents began fully 4 mm. anterior to the animal, were drawn past the sensory hairs into the buccal cavity, then were caught and turned to either side by the extended peristomium.

These currents serve a twofold function. They carry particles of food material into the region of the mouth, and also enable the animal, by means of sensory hairs, to test the media into which it is moving. These cilia always beat in the one direction. As the animal moves along, the anterior segments of the body are shifted about in all directions by muscular activety. The head is also advanced and withdrawn successively. With these movements are seen various foldings or puckerings of the prostomium in which the little sensory bristles seem alternately to be covered over and then extended through the loose enfolding membrane. These associated movements are designated as the "exploring reaction." Similar movements on the part of the flat-worm, were called "feeling movements" by Pearl ('03). At the suggestion of Dr. Jennings we have decided to classify these phenomena as "exploring reactions" as being less subjective and more appropriate in this particular field.

When approaching an unfavorable locality the "exploring" reaction becomes more pronounced, and is usually followed by a quick dart of the head backward and its extension in another direction. These darting movements always follow when the prostomium first comes in contact with any solid substance, or even with the surface film when crawling up the sides of the jar.

If the object is not harmful the prostomium may be returned and thus touch the object two or three times until a certain "familiarity" is established. The animal then moves over about the object without further retardation. If the object should be injurious the backward move is vigorous, the head is thrust energetically in another direction, and a general movement away from the scene of contact is made. Sometimes the avoiding movement is so energetic that the dart backward and toward one side are practically simultaneous. In this case there is usually a compensatory movement of the posterior segments in the opposite direction from that of the prostomium. At other times the action of the head may be entirely reversed by the violence of the movement and the animal will glide directly away from the unfavorable region. This movement is well illustrated when the prostomium comes in contact with one of the tentacles of a hydra.

The locomotor movements of Æolosoma while feeding are of a slow crawling nature. The beat of the cilia under the prostomium seem to play no part in them. Progress is made by means of successive alternate contraction and extension of the muscles of the body segments. In this way the Æolosoma may move backward or forward. In the more rapid of these movements the pharynx and papillæ are alternately stuck to the sub-stratum while the other end is advanced or retracted. This movement may be so vigorous as to resemble the looping of a "measuring" worm.

There is generally associated with the feeding reaction a certain peristaltic movement of the body wall which is entirely independent of the movements of the digestive tract. The wave begins in the region of the pharynx and runs backward to the end, slightly elevating the several bundles of setæ as it passes through them. There is no evident shortening or elongation of the body during the process. The waves follow each other in rhythmical succession at brief intervals. They may vary in size within rather wide limits. They are more vigorous about the time of the excision of a zooid from the parent. As constriction proceeds there is a noticeable "twitch" as the peristaltic wave passes from the parent to the zooid owing to the imperfect muscular connection

between the two. It is during one of these twitches that separation is finally affected.

If the food becomes scarce in the immediate neighborhood Æolosoma may glide or swim to other regions in a way similar to the gliding movement of a triclad. In this case, the cilia under the prostomium are the only organs of propulsion. The body is somewhat extended and straight. The peristomium is withdrawn so as to be practically invisible. The cilia under the prostomium seemingly beat more rapidly than before and propel the animal forward. The setæ are turned to a sharp angle caudad. Movement is always forward and may attain a speed of 5 mm. or more per second. All changes in directions are made by rotation of the head by exercise of the muscles of the pharyngeal region. When Æolosoma settles down after gliding or swimming the posterior segments are generally the first to come in contact with the substratum. Swimming in the open may also be stopped by a quick forward thrust of the setæ. This may be vigorous enough to stop all forward progress or to even throw the animal slightly backward.

When Æolosoma comes to rest where currents of water are flowing, its position is maintained by gripping the substratum with the pharynx in a way similar to the suction disc of a leech, as noted by Beddard ('88), or more generally, by sticking to it by means of the caudal papillæ. When feeding, under such conditions, the papillæ only are used, changes in position being made by looping.

The Æolosoma show a great tendency to burrow in the ooze at the bottom of the jar. The prostomium is narrowed laterally and extended somewhat in front and then thrust downward into the sediment. Progress is made by means of a rapid spiral twisting movement of the body.

In the performance of all these movements the animals secrete considerable mucus. The mucus entangles large numbers of bacteria and algæ which are caught up as the animal moves about. For this reason the Æolosomea will frequently twist about and browse over the mucus film surrounding the major part of the body, or apparently feed anew on old excreta. They are attracted

to the mucus tracts and excretory masses more largely when the food supply is scarce. Under such conditions, when one Æolosoma comes in contact with another the "feeding reactions" are mutually exhibited. They also get more or less stuck together by the adhesive mucus. If several get stuck together in this way the difficulties of separation become serious. This leads to what has been termed a "grouping" of Æolosoma.

The secretion of mucus may be also of protective significance. An Æolosom a was seized near the middle segments by one of the tentacles of an hydra. Immediately there was a violent end to end contraction of the body wall, in which large quantities of mucus and numerous oil globules were extruded. This stuff seemed to thicken considerably upon contact with the water. In a few moments the Æolosoma was able to squirm away with a spiral stretching movement leaving the mucus and globules in the grasp of the hydra. This heavy extrusion of mucus and globules was probably stimulated by the stinging cells of the hydra, as similar reactions are readily obtained with strong acid or alkali stimulations. These phenomena closely resemble the discharge of trichocysts by paramœcia under chemical stimulations (Massart '01); or when attacked by Didinium (Mast '09). Both of these authors assume that the trichocysts function as organs of defense.

Æolosoma come out more actively for food during the night. Many may be seen in the early hours of the morning feeding about the sides of the jar. On a bright day, when the jar is not protected from the light, they gradually seek refuge among the debris at the bottom of the jar. If the jar then be carefully covered over, in a few hours they come out of their hiding place and feed as before. When they are put into a clear glass dish on the stage of the microscope and bright light turned upon them from beneath they move about so actively that it is most difficult to observe them carefully. If a blue glass is placed beneath them and the source of light their movements are noticeably accentuated. Ultra-violet light ($\lambda 275$) destroys them in a few moments. Little change in their behavior, if any, was induced by red light. If a number of Æolosoma are placed in a clear glass dish and subjected

to very bright daylight for several days, the number and size of the oil globules are considerably increased. Budding and fission. however, show a marked retardation. On the other hand, if they are kept in the dark for the same length of time, the appearance of the oil globules undergoes no perceptible change, but the number of budding zooids will be noticeably large.

The activity of Æolosoma also varies with changes of temperature. When the water is cold their movements are correspondingly sluggish. As the temperature is raised the animal becomes increasingly more active until Ehrenberg's description as "extremely agile" becomes peculiarly fitting. The same animals will pass through all stages of relative activity from the early hours of the morning when the water is cool to the mid-afternoon when the temperature is raised by the warmth of the day.

These variations in activity are correlated with changes in the processes of constructive metabolism. If the animals are kept in cold water they feed very slowly and evidence of reproduction practically disappears. Just the reverse is true when the water is kept warm for some days.

Æolosoma seems to require certain periods of rest. The resting stage may be observed more generally when the light is subdued, and the water is cool. The only movement in evidence at such times is the gentle waving of the setæ. The animals lie somewhat extended and approximately straight. If left undisturbed they may lie in this position for several hours at a time.

Summary

The Action System

The daily life of Æolosoma exhibits, as has just been shown, certain movements which we may call the "action system" of the animal (Jennings '04).

Some of these are primarily concerned in the quest of, and the ingestion of food.

1 The beat of the cilia under the prostomium which may draw sample currents of water past the sensory hairs, or may propel the animal in gliding or swimming.

2 The "exploring" movements, which consist of little side thrusts of the head in various directions associated with a puckering of the prostomium.

3 The crawling movement, in which progress is made only by muscular contraction and extension. Progress may be assisted by alternate holding to the substratum by means of the pharynx and caudal papillæ.

4 The feeding reaction, which consists of the extrusion of the pharynx, and the swallowing of food particles.

Other movements are protective.

1 The avoiding movements, which consists of a backward dart of the head and its projection in another direction.

2 A vigorous contraction of the body segments which squeezes out many gland cells, mucus and globules.

3 Burrowing in the sediment.

4 Holding to substratum by means of pharynx or caudal papillæ.

More or less associated with all of the above movements are peristaltic waves and the constriction of zooids.

The Physiological States

The emphasis with which these movements may find expression and their coördination in the animal economy varies widely under changing conditions. These changing conditions in the ambient medium have an immediate effect on the equilibrium of the internal states of the animal. These physiological states, as associated with changes in external conditions, for our present purpose, may be grouped under the following categories:

1 *State of Relaxation.* This state is peculiar to a cool environment when the light is subdued.

2 *State of Normal Activity.* This embraces the phenomena of the feeding and exploring reactions, the crawling and gliding movements. It marks the time when the processes of metabolism and anabolism are in balance.

3 *State of Tension.* This marks the period when destructive forces are in ascendency. The movements are energetic and

exhausting. Readjustment of equilibrium through movement approaches a minimum. May be produced by high temperature or excessive light.

MATERIAL FOR EXPERIMENTS

Two species of Æolosoma were used in the course of these experiments. One, probably A. quaternarium, Ehrenberg, was found in great abundance in old paramœcium cultures. The basis of these cultures usually consisted of partially decayed leaves and grass from near the shore of a small pond in the Botanical Gardens of the University of Pennsylvania. The fact that the materials from which these cultures arose was gathered when dry suggests either that the Æolosoma come from encysted animals or from eggs. I have not been able to discover whether they come from one or both. The life history of this species seems to undergo periodic changes (Vedjovsky, 1892), quite different from that of A. tenebrarum. The cultures were always several weeks old before the Æolosoma appeared. When first discovered they always were full grown with the fission zone of budding zooids well marked in numberless individuals. From these same cultures after extensive proliferation for two or three months, the whole colony of Æolosoma sometimes disappeared in the course of a single night. I examined the sides of the jar and much of the débris at the bottom but could not find anything that was suggestive of the causes or results of this phenomenon. Beddard ('92) had the same experience but later discovered the encysted Æolosoma. He attributes the encystment to the approach of cold weather. My cultures evidently suffered from the introduction of some pathogenic conditions which destroyed them.

The other species, probably A. tenebrarum (Vedjovsky) was gathered in large quantities from the slime on loose stones along the shores of the Schuylkill River, just below Flat Rock Dam, a few miles north of Philadelphia.

These species, with a number of slime covered stones were preserved indefinitely in 8 inch battery jars. Other cultures of A. tenebrarum were frequently secured by placing old water hya-

cinths in battery jars filled with fresh water. In a few days large numbers of the Æolosoma might be observed during the early hours of the day, crawling about the sides of the jars.

There were a number of suggestive differences noted between these two species in their reactions to changes in the relative amount of light, moisture, and temperature pervading the ambient medium. These phenomena will be considered in another paper. At present our purpose is to work out the main features of the action system as shown in both species under the influence of external stimuli. We examine first the reactions to chemicals for the reason that with them it is easy to control and stimulate all the movements normally exhibited in the action system, and thus to observe the mechanism of the response.

REACTIONS TO CHEMICAL STIMULI

Methods

A graded series of experiments was made with the following chemicals:

1 Mineral acids: HCL, H_2SO_4, HNO_3

2 Organic acids: $HC_2H_3O_2$ (acetic), $H_2C_2O_4$ (oxalic), $H_3C_6H_5O_6$ (citric).

3 Hydrates: KOH, NaOH.

4 Carbonates: K_2CO_3, Na_2CO_3.

5 Halides: KCl, KBr, NaCl, NaBr.

6 Sulphates: $FeSO_4$,$CuSO_4$, $ZnSO_4$.

The acids and alkalies were titrated to standard normal solutions (n). The salts were prepared in gram-molecular solutions (m). The first series of experiments were made by allowing the chemical stimulus to flow toward the animal through a fine capillary pipette, the conducting tube of which had an inside diameter of 0.3 mm. the bulb holding about $\frac{1}{2}$ cc. of the fluid, with an air tube a trifle larger than the conducting tube. The orifice of the conducting tube was placed about $\frac{1}{2}$ mm. from the part to be stimulated. Localized applications of the chemical were thus made at the head segment, at the caudal segment, and finally upon the middle segment of the body.

A control with distilled water preceded each experiment. I found that the distilled water had to be particularly pure or of itself it would afford a definite stimulus. The water finally used was so prepared that the Æolosoma were practically indifferent to its presence in the control experiment. There is always a slight rheotactic stimulus to which Æolosoma will respond if the flow from the capillary tube is rapid enough. I found, however, that with the tube above mentioned there would be no characteristic response under the conditions stated. The chemicals were diluted in distilled water previously tested in the control.

In order to obviate, as far as possible, differences in reaction occasioned by such changes in the environmental conditions as variations in temperature and light would produce, the major experiments of this paper were performed during the early hours of the day, the culture jars being well protected from the light during the hours preceding, and the room shaded during the course of experiments. The temperature of the cultures and of the materials used was maintained very close to 15°C. Even with these precautions there is considerable variation in the behavior of different individuals preventing a precise quantitative test of stimuli. The qualitative reactions of many individuals, however, show a close correlation with various strengths of stimuli, so that we are able to analyze them with reasonable certainty.

In the course of these investigations several hundred experiments were made with each reagent. Only those, however, that have a direct bearing on our problem will be reported this time. I have therefore arbitrarily assorted the stimuli used into three main groups as shown in Table I.

TABLE I

	Threshold	*Normal*	*Strong*
Mineral acids	c. N/3000	c. N/1000	c. N/300
Organic acids	c. N/2000	c. N/600	c. N/200
Hydrates	c. N/1500	c. N/800	c. N/200
Carbonates	c. N/1200	c. N/500	c. N/200
Chlorides	c. M/80	c. M/40	c. M/20
Bromides	c. M/50	c. M/30	c. M/10
$FeSO_4$	c. M/10000	c. M/800	c. M/400
$CuSO_4$	c. M/80000	c. M/20000	c. M/1000
$ZnSO_4$	c. M/80000	c. M/20000	c. M/2000

Under threshold stimuli are grouped those solutions which lie at the threshold of physiological discrimination. They represent the weakest solutions which, under the conditions above stated, will interrupt the physiological poise of the animal at the moment of impact, enough to produce a visible reaction. They produce this effect only when introduced to the sensory hairs of the prostomium.

Under normal stimuli are grouped those solutions which will stimulate a characteristic reaction when introduced to any part of the body. They are not strong enough, however, to inflict any permanent injury to the tissues of the body. The movements occasioned by those reactions are seemingly normal. They have that easy flexibility which the animal daily exhibits under conditions favorable to its existence.

Under strong stimuli are grouped those solutions which force a powerful reflex movement on the part of the organism. The reactions are energetic and exhausting. They quickly develop fatigue, and upon repetition may prove fatal.

The concentration of the above solutions in each respective group is not exclusive. They vary rapidly toward or from each other under changing conditions. In our experiments, however, they were typical stimuli of the reactions now to be described.

The "Threshold" Stimuli

The threshold reactions were tested from a point directly in front of the prostomium and then lateral to the same.

Mineral Acids. When stimulated from in front there is a slight wrinkling movement of the prostomium, during which the head is advanced toward the pipette, then withdrawn and moved about with the characteristic exploring movements. In the case of H_2SO_4 these movements were repeated more vigorously than with the others, with the result that the head was soon brought into close contact with the pipette. There then followed a quick reflex away from the source of stimulus. With a lateral application the head is rotated toward the source of stimulation through

the pipette, after which the exploring reactions were exhibited as before. The animal then moves away at varying angles.

Organic Acids. The reactions were similar to the above. Acetic acid always seemed further to stimulate an erection of the more anterior setæ. The reactions to citric acid were similar to those with sulphuric acid. The puckering of the prostomium was more marked with oxalic acid.

Hydrates. It is very difficult to get a characteristic reaction with the hydrates. The animals invariably turn away from the source of stimulation without giving marked evidence of the exploring reaction. If the stimulus is repeated several times they curl up and give no further movement. When left alone they slowly resume normal activities.

Carbonates. The animal gives a positive reaction in every case. The exploring movements were well marked, and were associated with contractions of the pharynx as though feeding.

Halides. The head is swayed slowly from side to side with a rythmical motion. The peristaltic movements are markedly accentuated by both the Na and the K solutions. The exploring reaction was not in evidence. The animals make no effort to move toward or away from the pipette.

Sulphates. There is a positive reaction to $FeSO_4$ in every case, associated with the normal exploring movements of the anterior segments. $CuSO_4$ develops in Æolosoma reactions practically identical with those of the mineral acids. With $ZnSO_4$ exploring reactions are very slow, but the puckering of the prostomium is more strongly marked.

In all of these experiments, with the exception of the halides and hydrates, it must be noted, that in the stimulations lateral to the prostomium the animal first turns its head toward the side which is stimulated. The normal exploring reactions were then exhibited with the result that the animal finally moved toward or away from the field of stimulation.

Toxicity of Stimuli

Were these movements correlated with the relative toxicity of the elements used? To test this a number of Æolosoma were

placed in the several threshold solutions. It is obvious that immersion in these solutions presents to the animal membranes a more concentrated form of the chemical than they experienced under a localized impingement introduced through the pipette. After noting carefully the flow of the current from the pipette to the animal, which can readily be seen under the microscope, however, I am inclined to believe that differences in concentration due to diffusion are not significant. The ratio between the various stimuli will hold in either case.

When placed in N/3000 mineral acid solution the animals soon developed increased peristalsis and exhibited various twisting and stretching movements. They seemed to be normal on the following day. In N/2000 these movements were accentuated. The animals died within a day or two. A similar experience followed the use of N/2000 and N/1000 of the organic acids. N/1500 of the hydrates was fatal in four or five days. The Æolosoma, however, seem to be able to live indefinitely in N/1200 carbonate solution. The threshold solutions of the halides were fatal in a day or two. N/10,000 $FeSO_4$ was not fatal in four or five days. N/5000 was fatal in a few hours. The threshold stimulations of zinc and copper sulphates are not injurious to the animal. N/40000 of the zinc solutions, however, is fatal within 24 hours, that of the copper not within two days.

The evidence furnished by these facts in not very consistent. From the point of view of a positive or a negative reaction to a localized stimulus, however, we conclude that the reactions of Æolosoma to the threshold solutions of these chemicals may be correlated with their relative toxicity.

Nervous System and the Stimuli

Does the presence of a nervous system play a significant part in threshold reactions? To this test two series of experiments were used. In the first case the head segment was severed from the rest of the body with a pair of sharp needles. The cut surface healed over in a very short time. After several hours the above threshold experiments were introduced with negative results.

On the following day, when the new prostomium seemed tolerably well developed, there was still no response. About two days later, when the sensory hairs could be distinguished, the great majority of the animals reacted as normal.

The next experiment was made by tapping gently with a fine bristle that part of the epidermis in which the cerebral ganglia lie buried. The animal wriggles energetically at first, but upon repetition the nervous system suffers paralysis or fatigue, and the animal soon curls up and refuses further movement. After the Æolosoma had begun to relax, the threshold experiments were tried and received no response. In many cases it was nearly an hour before the exploring reactions could thus be stimulated. In all of these cases, very soon after the introduction of the mechanical inhibitions, the stronger solutions, here called "normal stimuli" would develop their characteristic reactions.

These data suggest that any interference with the integrity of the nervous system of Æolosoma, will raise the threshold of chemical stimulation.

Effect of Changes in the Ambient Medium on the Reactions

Changes in temperature were made by placing the culture dish in a water bath which is warmed or cooled to the required temperature and so maintained for several hours. The experimental media were also correspondingly treated. For very bright daylight the animals were subjected to the light coming from above and also reflected upward from beneath. For much of the time direct daylight was used.

1 If the temperature of the water is lowered to about 10° C. no characteristic response can be developed by means of these threshold solutions.

2 If the temperature of the water is raised to 20° C. the reactions to the above threshold solutions are very similar to those recorded later under the so-called "normal stimuli." The threshhold of chemical discrimination is raised to solutions more than twice as dilute as the above.

3 If the animals are subjected to bright light for several hours

and then treated with these weak solutions the results are similar to those produced when they are subjected to a raise in temperature, although the threshold varies within much narrower limits.

The "Normal" Stimuli

Reactions Under Uniform Conditions

Mineral Acids. When solutions of this strength are applied to the tip of the prostomium there is first evident a wrinkling of the prostomium and an erection of the setæ of the anterior segments. The head is then drawn backwards and quickly turned toward one side. Sometimes the negative reaction may be so marked as to reverse the general direction of its movement prior to stimulation. As a rule the animals move at varying angles away from the field of stimulation. When applied laterally to the prostomium the head segment is frequently turned toward the pipette before the negative reaction is expressed. Stimulation at the posterior

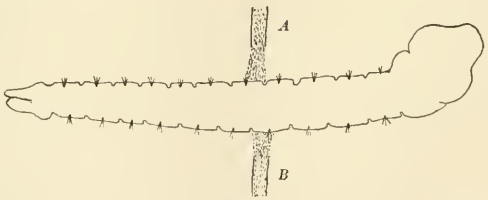

Fig. 2

papillæ causes the posterior segments to contract, thus pulling the papillæ forward. Sometimes the caudal segment is swung away from the stimulus. When the chemical is first applied at the caudal segment there is no change in the attitude or movement of the anterior segments. Repeated stimulation may cause rapid crawling forward.

When the chemical is applied to the middle segments of the body the ventral muscles always contract more than the dorsal. The resultant movements are determined by the attitude of the animal at the moment of stimulation (Fig. 2). If this attitude is

like that shown in Fig. 2A, the first contraction throws the head nearer to the stimulus. This is followed by a reverse movement in which the animal moves away. If as in Fig. 2B, the first contraction turns the animal away from the stimulus and movement is continued in that direction. This principle holds true regardless of the direction from which the pipette may throw the impinging chemical upon the body wall,—whether it be directed toward either side, dorsally or ventrally. The "secondary reflexes"—those movements which immediately follow this first reflex—are such as to move the animal away from the field of stimulation.

Organic Acids and Hydrates. Reactions to these solutions were similar to those developed by the mineral acids. With the hydrates, however, it was noticed that many individuals would stop in their onward rush from the stimulus, would curl up in crescent shape, and seemingly rub the prostomium to and fro with a lateral swing on the substratum. When placed in a culture jar after the experiment they invariably burrowed into the ooze at the bottom of the jar.

Carbonates. There are quick negative reactions to the carbonates at both head and caudal segments, though the animal makes but little effort to get away from the stimulus. When stimulated at the side so that the ventral contraction throws the head nearer to the pipette, in few cases was there any evidence of a reverse movement. Vigorous puckerings of the prostomium were noticeable; a few writhing movements were made; and then the animal would usually lie dormant until the effect of the stimulus passed away.

Halides. The initial reaction to the halides was like that to the acids. These were invariably followed by so marked an increase in the peristaltic waves, however, that progressive movements were inhibited for some time. The K solutions stimulated increased peristalsis much more vigorously and quickly than the Na solutions.

Sulphates. When $FeSO_4$ is introduced directly in front of the animal, the head is slowly waved to and fro, laterally, several times, after which the Æolosoma curls up with a twisting motion, and makes no effort to move away. When introduced laterally the

head is first turned toward the pipette before expressing these movements. There is a large increase in the amount of mucus secreted. When stimulated at the posterior papillæ, the caudal segments are slowly contracted and swing toward either side. Some seconds after stimulation the head segment shows a marked increase in the exploring reaction. When applied to the lateral segments the animal first curls as usual, then twists about with increasing energy but with no locomotor results. The reactions to $CuSO_4$ were similar to those with the mineral acids. In the case of $ZnSO_4$ the great majority moved away from the stimulus with a spiral twisting reaction, alternating this reaction every few seconds by curling up and vigorously rubbing the prostomium on the substratum. When put back into a culture dish they immediately burrowed into the ooze at the bottom of the jar.

In all of the above reactions, which were followed by locomotion there was noticeable a marked increase in the vigor of the characteristic "exploring movements." The end result was that the animal followed a decidedly zig-zag course.

Reactions Under Changing Conditions

a. The head is severed from the body by a cut through the region of the œsophagus. Within a few hours the anterior segment of the body will respond to the above stimuli of normal strength in a way characteristic of the normal exploring reaction. Lateral applications may cause the dorso-ventral contractions but they are not followed by the reverse movements that are noted under normal conditions.

b. The animal is physiologically depressed by tapping the cerebral ganglia as described under threshold stimuli. The reactions to the various chemicals under these conditions do not exhibit the usually distinctly negative quality but resemble more closely those given above under $FeSO_4$.

c. The temperature is gradually lowered to about 10° C. It is difficult to get any characteristic reactions except at the prostomium, and these correspond closely to the threshold reactions at 15° C. The lack of any marked reaction when the body segments

are impinged upon by the chemical is evidently due to the formation of a protecting membrane or the thickening or hardening of the mucus film which surrounds the body, under the stimulus of the cold.

d. The temperature is raised to about 20° C. The reactions to these solutions are quick, almost violent. When the chemical is applied directly in front of the prostomium the reaction invariably brings about a complete reversal of the line of movement. When applied laterally to the prostomium the reaction is directly away from the pipette without preliminary testing movement. When applied to the middle segments of the body the contraction is so vigorous that in many cases oil globules and gland cells are squeezed out in the process. In the case of the alkalies there was noticed the beautifully rich magenta coloring observed by Beddard ('89). Application at the posterior papillæ readily stimulated the forward crawling movement even to "looping."

e. The animals were subjected to very bright daylight for several hours.

With the water at a temperature of 10° C. many of the reactions were in accord with the original experiments at 15° C. With the temperature maintained at 15° C. the reactions were much more vigorous and largely resembled those expressed in response to the stronger solutions. Many of the contractions were vigorous enough to squeeze out oil globules and mucus cells. With the temperature raised to 20° C. the reactions were vigorous and exhaustive, and in many cases proved fatal.

The "Strong" Stimuli

Reactions Under Uniform Conditions

Mineral Acids. When stimulated at the tip of the prostomium there is a quick negative reaction which may reverse the direction of movement. Sometimes the head is thrown only part way toward the rear and the animal moves away at an angle. The progress of the stimulus can be noted. The prostomium is first bowed away from the stimulus; all the setæ are erected rigidl at

right angles to the body, then a sudden turn is made away from the stimulus. With a lateral application to the prostomium the head is not turned toward the pipette before the negative reaction takes place. Application at the posterior papillæ stimulates a quick contraction of the posterior segments and a rapid crawling movement forward. Lateral body stimulation follows the same rules as under normal stimuli, only the reactions are far more energetic. Not infrequently the first contraction will throw out many oil globules, etc. Movement away in this case is through a spiral twisting reaction.

Organic acids were similar in effect to the mineral acids.

Alkalies. All reactions were in the shape of energetic contractions of the body, away from the stimulus when applied to the ends of the body, with the bulge towards the stimulus in response to the lateral exposure. The animals were powerless to make any further effort to leave the field of stimulation although they recover rapidly from the shock. The muscles of the body wall facing the pipette seem to be paralyzed by the chemical as that side seems passive in the movements that soon follow. These movements are an alternate contraction and extension of the muscles of the body wall opposite to the place of stimulation.

Halides. There is a prompt negative reaction when these are applied to the head and the animal is usually able to get away from the stimulus. This is not the case when the caudal end or side is stimulated. When stimulated at the side the first contraction is followed by increasingly large peristaltic waves running from pharynx to papillæ, which effectively inhibit any locomotor movements. With the bromides at the papillæ, the papillæ are quickly stuck to the substratum while the anterior parts twist and writhe in all directions. With the chlorides a forward contraction may advance the animal a little, but it soon loses power of orientation and lies at the mercy of all sorts of muscular contraction and extension.

Sulphates. The reactions to $FeSO_4$ were similar to those with the halides. $CuSO_4$ and $ZnSO_4$ had the same effect as the acid solutions.

All of the chemical solutions under this category had the further

effect of producing precocious excision of budding zooids. Many of these zooids were so immature that the budding zone was almost imperceptible, yet they were readily snipped off by the energy of the contraction, or through the agency of the accentuated peristalsis. They usually survived the shock, and were able to swim about on the following day. In many cases where a repetition of the stimulus proved fatal to the parent stem, these prematurely excised zooids recovered.

Reactions Under Changing Conditions

With head segment removed all of the characteristic reflexes were given. I was able to stimulate the crawling movement by application of the chemicals at the papillæ. This was also true after the animal was depressed by tapping over the cerebral ganglia.

When the temperature of the water was lowered to 10° C. the reactions took more the form of those given under normal stimuli. The animals endured repetition of the stimulus without fatal results. With the temperature raised to 20° C. it was difficult to apply the stimulus to all without the reaction being so vigorous as to prove fatal. This could only be done when the animal was stimulated at the prostomium. In these cases the negative reaction is so vigorous, especially with the acid, the copper and zinc sulphate solutions, that the animal is thrown directly away from the source of stimulation, and far enough so as to be able to escape.

If the animals were subjected to very bright daylight before these experiments were made the solutions invariably proved fatal soon after the first application of the chemical. The influence of the light upon the tissues of the body is such that it increases the toxicity of these solutions if they are applied after the animal has been exposed to the light for a few hours.

SUMMARY OF RESULTS OF THE EXPERIMENTS

These experiments show that every movement expressed by the action system of Æolosoma in its native environment may be reproduced under conditions of control through the agency of various chemical stimulations.

Results with "Threshold Stimuli" (chemicals very weak, see p. 56)

a Chemical stimulations of threshold intensity develop the normal exploring reaction in Æolosoma.

b If the stimulus impinges laterally to the prostomium, there is a turning of the head segment toward the field of stimulation before the exploring movements are expressed.

c After a brief exploring reaction the animal moves toward or away from the field of stimulation—gives a "positive" or a "negative" reaction to the stimulus.

d The movements expressed in these reactions vary within rather wide limits, and cannot be coördinated with "lines of diffusion."

e The aggregate of movements exhibited varies with changes in the chemicals used. The nature of the stimulus is an important factor in determining the nature of reaction.

f Any interference with the integrity of the nervous system raises the threshold of chemical discrimination.

g The reactions to threshold stimuli may be loosely correlated with the relative toxicity of the chemical involved.

h The threshold of chemical discrimination varies rapidly with changes in the physical nature of the environment.

Results with "Normal Stimuli" (chemicals moderately strong, see p. 60)

a Æolosoma may exhibit all of the movements comprised in the action system in response to chemical stimulation of this order.

b There are no characteristic positive reactions to chemicals of this order.

c Many of the negative reactions are directly away from the field of stimulation. The great majority of the negative reactions, however, are composed of decidedly random movements, which continue until the animal is freed of the stimulus.

d There is a certain degree of individuality among the segments. The posterior segments may be made to give a definite

reaction without any response being given by the anterior segments.

e Different parts of the body are affected in different ways by the same stimulus. A given stimulus which may cause a well coördinated negative reaction if applied at the anterior segment, may inhibit coördinated movement if applied at the middle segments of the body.

f Any interference with the integrity of the nervous system seriously interferes with the power of coördinated movement.

g Physical changes in the environment, due to variations in the relative amount of light and heat pervading it, produce an effect upon the animal economy equivalent to the effect produced by different concentrations in the chemicals used in these experiments.

Results with Strong Stimuli (chemicals very strong; see p. 63)

a Reactions to chemicals of this order, when applied to the anterior end consist of a vigorous reflex movement which throws that end away from the field of stimulation. The animal may then exercise its powers of locomotion and escape.

b When these chemicals are applied to any other part of the body the reflexes are of such a nature as to inhibit coördinated movement away from the stimulus.

c Interference with the integrity of the nervous system does not seriously modify the type of reaction developed by these solutions.

d The energy imparted to the animal by these chemicals may be directly accentuated by an increase in the relative amount of light or heat pervading the ambient medium.

e The relative toxicity of these solutions depends largely upon the age of the part impinged, and also upon the physiological condition of the organism as a whole.

CONCLUSIONS

By means of the localized application of different chemicals with varying concentration we have stimulated in Æolosoma characteristic movements designated as the action system. This shows

conclusively that chemotoxis plays a very significant part in the methods and processes of animal behavior. It shows, further, that under conditions of control one may approximate the physiological states which underlie the movements an organism may exhibit in a state of nature. The various movements which we have stimulated artificially, are of the nature of reflexes, more or less complex. Many of these are so haphazard in their expression that they seem to be merely the spontaneous play of various amounts of energy, released within the mechanism of the animal. On the other hand, some of these movements possess a certain element of precision or adaptation which is manifestly beneficial to the organism. They remove the organism from an injurious environment in the quickest possible way. One of the first problems in Animal Behavior is: How did these more adaptive reflexes arise in a state of nature? Our effort, therefore, will be to correlate the reflexes observed here under wider categories that will help to interpret the action system of Æolosoma in a phylogenetic way. Before we suggest a solution of our problem, however, it is necessary to estimate carefully the modus operandi of the various reactions involved.

In the case of the threshold reactions, when the stimulus impinged laterally upon the prostomium there followed a turning of the head segment toward the source of stimulation. Was this turning due to the asymmetrical impingement of the lines of diffusion, or to the electrolytic effect of moving ions upon the cell membranes of that side of the head? In some way both of these factors may have been involved. On the other hand it must be noted that although the animal turned toward the side which was stimulated, the angle in which the solution is projected toward the animal through the pipette may vary within enormous limits without developing any variation in the side thrust of the reaction which immediately follows. The prostomium is turned toward that side which is first impinged upon by the chemical, regardless of the direction from which that chemical may come. Again, after the initial exploring reaction has been expressed, with the exception of the positive reactions, all of the succeeding movements have no direct reference to the stimulus, its direction, or its source.

The fact that only experiments with electrolytes are recorded here suggests an interesting problem from that point of view. Several hundred tests were made with the non-electrolytes—urea, cane sugar and glycerine,—but with negative results. An effort is now being made to find a non-electrolyte that will stimulate a characteristic reaction, similar to any of the above. After these experiments are concluded, something further may be determined as to the role of electrolytes in this field. The fact that Pearl ('03) stimulated a similar turning of the anterior part of the flatworm—equivalent to our exploring movement—by means of a light touch with a piece of wood, suggests that the secret of this reaction lies within the confines of the animal economy. We therefore, conclude, for the present, that the turning toward the stimulus in the case of Æolosoma, is due to a sense of chemical change; to a difference in intensity arising locally in the animal's environment. The movement was not an orientation.

Associated with this turning of the head the exploring movements were expressed. The animal then moved toward the source of the chemical, exhibiting the feeding reaction, or away from the same by means of a slow crawling movement. The positive reactions were due to the fact that the weak solutions of the carbonates or iron sulphate stimulated certain internal changes akin to those induced by food particles. It is interesting to note that with a rise in the temperature of 5° C., or more, the animal uniformly responds negatively to these stimuli. The ability of these solutions to stimulate the feeding reaction is thus conditioned by the physiological state of the organism—by its previous internal reactions to external changes in its environment.

As the concentration of the various chemicals is increased, the reflexes become more and more distinctive. When the chemical is applied to the prostomium the reflex is always lateral—away from the impinging stimulus. This is usually a right angled turn in the case of "norma." stimuli. If the chemicals are much stronger than this grade, two facts are noticeable. In the first place, the reactions to prostominal stimulation are similar with all the chemicals. This is never the case with the weaker solutions. In the second place the axis of locomotion is directly reversed by

the force of the stimulated reflex. As the chemical becomes more and more dangerous to the organism, the initial reflex, developed upon contact with the chemical, throws the animal ever further away from its influence. Is this reaction due to an increase in control of the movements of the animal by the lines of diffusion, or is it because the vigor of the reflex is proportionate to the amount of energy liberated by the impinging stimulus? It seems most clear that the latter suggestion, only, can be adjusted to all the data here involved.

When the stronger stimulus is applied to the mid-sections of the body the reflexes are always ventral—irrespective of the exact locus of stimulation or the direction from which the chemical may come. This reflex tends to bring the head and caudal segments together. Sometimes this serves also to bring the prostomium nearer to the source of the stimulus. A counter reflex is then given, characteristic of the regular prostomial stimulation-reflex, which throws the anterior end away from the chemical, and if the stimulus is not too strong, the animal escapes.

The movements given in response to the different chemicals vary within very wide limits. Each group of chemical stimulates reactions peculiar to themselves. Throughout the whole series, the ability of the animal mechanism to adjust itself to the impingement of a chemical upon the body wall (excepting the anterior segment) varies inversely as the strength of the chemical. This phenomenon is practically reversed in the case of prostomial stimulations. In these cases we have what we may call a prostomial reflex which is inherently negative and which serves a fundamentally regulatory function as the animal approaches a marked change in the environmental conditions.

These facts show conclusively that the problem of animal behavior must look for its solution in the physiological arrangement of the protoplasmic aggregates of the organism under investigation. So far as external stimuli are concerned we may conclude:

1. The sort of stimulus does not predetermine the reaction that will follow upon its impingement upon any part of the organism, although it may contribute a significant thrust to that reaction.

2. The direction of the impinging stimulus—lines of force or of diffusion—affect the direction of the resulting reflex only incidentally. The morphology of the organism is the determining factor.

3. The intensity of an impinging stimulus, or variations in its intensity, are significant in so far as they interrupt the physiological poise of the organism involved. They may determine the vigor of, but not the sort of, reaction that may be expressed.

With these facts in mind, we may attempt an outline of the phylogenetic rise of the action system of Æolosoma in so far as it had been analyzed in this investigation. The basis of behavior rests upon the irritability of living protoplasm. A thoroughgoing interpretation of this irritability is yet to be made; it is far beyond the range of our present experimental knowledge of protoplasm. This much we do know—irritability presupposes movement, and the use of movement formulates the quest of "behavior." All of the movements potential to the protoplasmic aggregates, which we designate as an individual organism, are variously being expressed in the course of its life history (Jennings, '07). Some of these movements, in periods of stress, more readily than others, restore a certain physiological equilibrium, which is essential to the welfare of the organism, and which has been disturbed by the impingement of an external source of energy. Repetition of equivalent conditions of stimulation tends to reproduce such movements with increasing celerity, under the law of the readier resolution of the physiological states (Jennings, '04). By the process of natural selection these movements have been selected into a system of characteristic reactions which we designate as the "action system." The rise of an action system has further played a profoundly morphogenetic rôle in the course of history of the organism (Bohn, '06). The animal is what it is because of past behavior.

This brief outline is esentially a recapitulation of the "trial and error" theory of the rise of behavior as advocated by Jennings, or the selection of random movements as suggested by Holmes.

It attempts to balance the play of both internal and external forces in the rise of an individual animal economy. There

are a number of investigators, however, who insist that for the sake of a more objective interpretation of the facts of behavior, more emphasis must be given to the orienting force of the external stimulus. Loeb ('88) observing that the heliotropism of many animals was singularly akin to similar phenomena exhibited by plants, suggested that the orienting function of lines of force (lines of diffusion, 1903) expressed by the equation F (i), playing upon asymmetrical parts of an organism would account for the more precise movements exhibited concomitantly with the impingement of the stimulus involved; the "positive" or "negative" reactions. Bohn in a series of excellent papers, and many other investigators, in this field, have shown conclusively that the directive force of any reaction which follows any sort of stimulation can be predicted only by a knowledge of the play of the previous forces acting upon the animal economy; by a careful estimate of the arrangement of characteristic internal aggregates which Jennings denominates as the physiological states. A more intimate knowledge of these shifting aggregates called physiological states is certainly essential to any far-reaching interpretation of behavior. Our own data does not admit the classification of any of the movements of Æolosoma as "orientation" in the tropic sense of the term. Loeb ('97) appreciating this difficulty, added another factor in behavior which he called Unterschiedsempfindlichkeit; represented by the formula $F \frac{di}{dt}$. This factor, however, throws the interpretation back to the physiological states, which are included in our analysis as given above.

More recently Walter ('07), probably representing the view of a number of investigators, defines the theory of "tropisms" as essentially based upon "an asymmetrical reaction to an asymmetrical stimlus." Granting to this view all that he would include we seriously question whether such a comprehensive statement can throw much light on the problem of behavior. Any flexible movement in nature, whether exhibited by what we call a living object or a dead, may readily be adjusted to this category without acquiring any added significance thereby. There is a wide distinction between an interpretation of a reflex movement as a reac-

tion away from an injurious stimulus, or as an orientation by lines of force to bring about symmetrical impingement. Our experience with Æolosoma will not support the latter interpretation.

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SELECTION OF FOOD IN STENTOR CÆRULEUS (EHR.)[1]

BY

ASA ARTHUR SCHAEFFER

WITH TWO FIGURES

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INTRODUCTORY

The question whether protozoa can exercise selection in the kind of material which they feed upon has called forth expression of opinions from almost every worker upon the protozoa. The inges-

[1] From the Laboratory of Experimental Zoölogy, Johns Hopkins University. The author of this paper is indebted to Prof. H. S. Jennings under whose direction this investigation was carried on.

tion of insoluble material is readily observed in many of these organisms, and this fact together with the generally accepted notion that the protozoa are much simpler organisms than the recent work shows them to be, was perhaps largely responsible for thinking the selection of food a question answerable by more or less casual observation and by inference from the general behavior. This is brought out by comparing the views of some of the more important workers.

Prior to Verworn's work, which may be regarded as the point of departure for the recent interest in the protozoa, the consensus of opinion of workers in this field seems to have declared in favor of the ability of these organisms to ingest certain kinds of particles and to reject certain other kinds in a systematic manner. Thus Stein ('67) and Entz ('88) and others declared unequivocally that in infusoria, whenever "foreign" particles were brought by the "alimentary vortex" into the "pharynx," the current was stopped or given such a direction that the foreign particle was swept away. Neither of these writers, however, seems to have taken into account nor tried to explain the earlier observations of Ehrenberg ('38) and others, who described the ingestion by certain infusoria of large quantities of carmine grains, which can hardly be regarded as anything but "foreign" particles.

Verworn ('89) took up this question and confirmed Ehrenberg's results with carmine; he also observed that chalk crystals, indigo particles, and the like, were freely ingested. At the same time that these indigestible particles were swallowed, small organisms such as swarm spores and micrococci were often swept away by the cilla. From these and other observations Verworn concluded that there is no selection obtaining among the various kinds of particles which the alimentary vortex brings to the mouth of the infusorian.

Bütschli ('89) also came to the conclusion that the power of choice of food is absent in the protozoa.

But in 1893 the opposite view was again advanced by Hodge and Aikins, who said that "a prine condition of the creature's (Vorticella) life must be its ability to distinguish food from what is not food." But there is no reference to or explanation of either

Ehrenberg's or Verworn's work mentioned above, where indigestible particles were described as being freely eaten.

Jennings in 1902 worked upon Vorticella and confirmed Ehrenberg's and Verworn's experiments; he showed that Hodge and Aikins' conclusions were probably drawn from insufficient data. Similar experiments upon Stentor also showed that this infusorian ingested large quantities of carmine, india ink, etc. From these experiments Jennings came to the conclusion that these organisms probably do not have the power of selecting their food in any precise way.

In 1907 there appeared a preliminary paper by Metalnikow "Ueber die Ernährung der Infusorien und deren Fähigkeit ihre Nahrung zu Wählen," in which the author describes the taking up or ingesting of carmine and india ink by paramecium, but states that if left in water in which is suspended carmine or ink, the paramecia gradually take in less and less of these substances until in about 18 days few or none contain either ink or carmine. According to Metalnikow the paramecia are gradually "educated" in some way so that they cease after awhile to take the carmine or ink. This paper will be more fully discussed further on.

This brief historical account includes most of the more important references to the ability of protozoa to select food. These references are all incidental in character and the experiments upon which they were based were in almost every case few and not varied. This lack of experimentation was probably due to the notion that if they could discriminate at all, the protozoa should tell with precision for each and every particle whether it is food or not, and that any mistakes would be sufficient evidence that the ability to choose among particles of various sorts is absent. In short, machine-like accuracy seems to have been expected if selection is present at all.

But we can hardly with clear thinking demand more prefect selective faculties in the protozoa than in the higher vertebrates. If one should draw the conclusion that because one observes a horse eat bits of a weather-beaten fence rail, the horse has not the ability to select his food, the logic would be equivalent to that which is used when it is affirmed that because a protozoan eats car-

mine, the protozoan cannot express choice in food. The phrase "selection of food" is evidently vague, and great care is necessary in interpreting results when this concept is applied to specific instances. In this paper the words "selection," "choice," etc., are used only in a purely objective sense.

These considerations, together with the fact that most protozoa are observed to contain in their bodies only food materials in various stages of digestion, has led the present writer to carry out a number of experiments on this matter, using many substances, digestible as well as indigestible. Because of large size, transparency, sessile habit, and highly developed ciliary apparatus, the Blue Stentor (Stentor cæruleus Ehr.) was selected as affording probably the best opportunity for investigation. The question proposed, was: Does Stentor ingest all particles that reach its disk, or are swept into its pouch; or are some eaten and some rejected, depending on whether they are or are not good for food? This is the central question in this paper. A number of other matters were also dealt with, as: the existence of conditions of hunger and satiety; the basis of the selection, whether chemical or tactual, etc. These questions will be taken up at their proper places.

MATERIAL

The Stentors used in these experiments and the organisms used for food were collected from a number of widely separated localities, viz: Cold Spring Harbor, L. I.; Kunkletown, Pa., and various places around Baltimore. This was done to determine whether there were differences in Stentors that grew in different localities with regard to the power of selecting food. After it was found that Stentors from all localities and from laboratory cultures gave practically the same results, the larger part of the work was done upon specimens raised in the laboratory. All the organisms used for food were also raised in the laboratory, excepting Phacus and Euglena, which were always collected from wild cultures. The food of the Stentors raised in the laboratory consisted of bacteria and paramecia almost exclusively. Many of the Stentors which were used in the experiments where Euglenæ and Phacus were fed,

had not eaten any of these latter organisms prior to the experiment, nor had their ancestors for many generations eaten either Phacus or Euglenæ.

FOOD OF STENTORS

It is of course not always easy to determine what materials actually serve as food and what do not; this is particularly difficult in so minute an animal as Stentor. The application of tests for food value used with higher animals is quite impracticable. deciding whether certain things should be classed as food for Stentor, the following criteria were employed: (1) Long continued feeding of the substance in question must not injure the animal in any way. (2) The material must decrease in quantity in passing through the body, showing that some part has been absorbed. These two tests were applied with great care to the substances which Stentor was seen to eat. Tabulated results follow.

1 Substances eaten rather freely which do not serve as food:

Powdered carmine	Powdered india ink	Powdered charcoal
Dead yeast plants (?)	Raphidium	

2 Substances eaten only occasionally which do not serve as food:

Powdered glass	Fine sand	Powdered sulphur
Potato starch grains	Bits of detritus	

3 Substances eaten freely which serve as food:

Small Stentors	Paramecia	Chlamydomonas
Phacus triqueter	Phacus longicaudus	Euglena viridis
Euglena spirogyra	Euglena deses	Trachelomonas hispida
Trachelomonas volvocina	Stylonychia	Monostyla
Arcella	Coscinodiscus	Lyngbya
Oscillaria	Peranema	Chilomonas
Hydatina	Colpidium	Amœba
Halteria	Spirostomum	Bacteria

METHODS OF INVESTIGATION

There are two methods by means of which choice of food can be investigated in such an organism as Stentor.

One method is specific, consisting in observing and recording the path and fate of each particle that is fed to the Stentor. This is accomplished as follows: A capillary pipette is made by drawing out an ordinary pipette to a very fine hair having an internal diameter of about 75μ or less. Food particles, such as Phacus, Euglenæ, etc., or indigestible particles, as the experiment may demand, are then sucked up into the pipette with some water. Several Stentors are transferred from the original culture dish into a watch glass with a few cubic centimeters of the culture solution and placed on the stage of a binocular microscope of the Braus-Drüenr type. A magnification of about 65 diameters is used. After the Stentors have become attached to bits of detritus in the watch glass, the particles are fed from the pipette, the end of which is held very carefully about the diameter of the disk away from and above the Stentor's disk. The particles are for the most part fed successively, in each case waiting until the foregoing particle is swallowed before the succeeding one is set free from the pipette. Much time is of course taken up in the recording of results and in getting the pipette into position again for feeding. Some idea of the time required in making such feeding experiments may be obtained from the fact that a successful experiment in which 120 particles are fed extends over about an hour and three-quarters. When it was desired to feed two or more kinds of food, the particles were first mixed in the desired proportion and then sucked into the pipette. If the various sorts did not come in the desired order some of the particles were merely dropped to the bottom of the dish and not allowed to touch the disk of the Stentor at all. In this way the order of substances in a mixed stream was under control. Nothing was fed when it was not intended. With a method of this degree of exactness there seems to be no reason why the results should not be thoroughly reliable. The only considerable variant which seems possible is the physiologic state of Stentor—which it is the purpose of this paper to investigate.

While the method outlined above is the best possible for solving many of the questions that are bound up with that of choice of food, there are nevertheless other points which cannot well be cleared up in this way. For it is well known that Stentors eat and thrive upon such small organisms as bacteria, and it would be impossible for several reasons to note what happens to each individual bacterium as it is swept into the Stentor's pouch. For getting at questions of this nature there was employed another method which may be called an indirect method as compared with the one described above.

When it is desired to test the relative readiness with which very small particles such as bacteria, Peranema, yeast cells, finely ground carmine, etc., are taken up by the Stentor, it has been found that the best method is to mix up quantities of them in the desired proportion and then to introduce into this mixture some normal Stentors which have very little or no food in them. After a stated time the Stentors are taken out and squeezed under a cover glass in order to examine their contents. The most difficult point is to maintain in the mixturue the original uniform distribution of the particles. This difficulty was mostly overcome by frequent stirrings and by placing the dish in the dark to prevent reactions to light, as will be described more fully later. By the use of this method very important results have been reached which could not have been obtained otherwise. Each of these two methods acts as a check upon the other, and at the same time they verify each other's results.

THE NORMAL BEHAVIOR OF STENTOR

To understand the experiments bearing on the choice of food the reader should have a more or less clear idea of the normal behavior of Stentor. This subject has been dealt with to some extent by various observers, notably by Jennings, but in the course of my investigations several points have been cleared up that were heretofore more or less imperfectly understood. Several new features of behavior have also been discovered that play at certain stages essential rôles in the choice of food, and it is necessary

perhaps that these should be fully described at this point to avoid the necessity of giving an account of them while discussing the experiments.

In a normal attached Stentor in a watch glass under a binocular microscope there are observed four groups or systems of cilia by means of which the greater part of the behavior is effected. (See

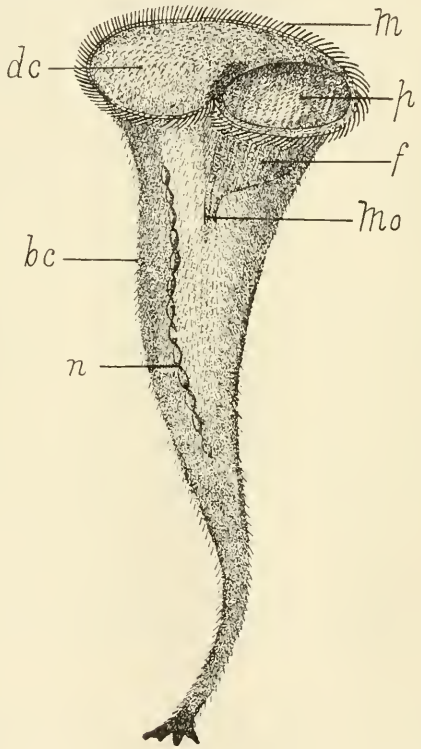

Fig. 1. Normally extended "hungry" Stentor. *b c*, Body cilia; *d c*, discal cilia; *f*, funnel; *m*, membranellæ; *mo*, mouth; *n*, nucleus; *p*, pouch.

Fig. 1.) These four systems are: (1) the membranellæ, (2) the discal cilia, (3) the cilia of the pouch and funnel, and (4) the general body cilia found on the sides of the Stentor. Each of these sets of cilia has a function quite different from that of any of the other groups, and the extent to which their behavior can be modified also differs among the various groups.

The most conspicuous of these ciliary appendages are of course the membranellæ, which are inserted around the rim of the disk. Their normal action creates the well known alimentary vortex by means of which a constant stream of water is caused to flow against the disk. In this manner the Stentor procures whatever small particles there may happen to be suspended in the water. The particles as well as the water in which they are suspended are driven against the disk more or less perpendicularly. The water is driven out over every part of the rim of the disk, while the particles striking against the disk are carried slowly by the discal cilia toward the pouch. The transportation of the particles on the disk is not due to any feature of the movement of the membranellæ, but is entirely due to the discal cilia. These are very small organs and are disposed in rows more or less parallel to the membranellæ. Their action cannot be observed except under the high power of a compound microscope. Their arrangement, size, etc., can then also be seen. The particles, as they strike the disk, are taken by these cilia and slowly passed on in the direction of the pouch, over the rim of which the particles are dropped. The particles are then taken by the pouch and funnel cilia and either passed down to the mouth, where they are ingested, or else are swept out over the rim of the pouch on the ventral side of the Stentor. The cilia of the pouch and funnel are considered as of one system because their functions are identical as far as can be determined. It has been found impossible to observe just how these cilia beat under various conditions. It is of course quite certain that when a particle is ingested they beat downward toward the mouth, and that when a particle is rejected they beat in the opposite direction. It is also found that a small particle in the center of the pouch is little acted upon by the cilia, but as it comes nearer the cilia it begins to travel faster. It is probable therefore that the transfer of particles in the pouch and funnel is effected more by actual contact with the cilia than by mere transportation in a current of water which is set in motion by their action, and this is probably true also for the action of the discal cilia. Only small particles are wholly transferred by the action of the pouch and funnel cilia. Large particles, such as paramecia, are ingested

by the combined action of the cilia and the compressive movement of the walls, of the pouch and funnel. The general body cilia play the least important part in the securing of food. They are distributed in rows over the surface of the Stentor included between the foot and the edge of the disk. Their chief function is that of locomotion. But when the Stentor is attached, their backward or footward beat helps to get rid of the water flowing over the edge of the disk which no longer contains food, and also carries out of reach of the vortex those particles which are dropped over the edge of the pouch in the mid-ventral notch. By this means the vortex always consists of water and particles which for the most part had not struck the disk before. The body cilia thus serve to increase the food-getting ability of the Stentor. This constitutes the normal action of all the cilia of a normal attached Stentor under usual circumstances when ingesting food.

BEHAVIOR IN REJECTING PARTICLES

The rejection of a particle is accomplished by various modifications of the ciliary movements outlined above, depending upon the strength of the stimulation. In the simplest case the rejection of a particle is produced by a reversal of the cilia in the pouch, or in the funnel, or in both. There is much variation even in this apparently simple method of getting rid of an objectionable particle. The course of the particle may be altered at any stage in passing from the interior surface of the pouch to the mouth opening at the bottom of the funnel. If the substance is a small sand grain, e. g., the course is in almost every case altered before the funnel opening is reached. That is, the sand grain is ejected by a strong, presumably outward, beat of the pouch cilia. The funnel cilia play no part in such an instance, as may be seen occasionally when there is a food particle in the funnel at the time there is a sand grain in the pouch. The sand grain is ejected while the food particles in the funnel are ingested. When the substance is one like a starch grain, or a grain of carmine, or a food particle when the Stentor is not "hungry," the course of the particle may be altered anywhere from the interior surface of the pouch to the mouth open-

ing. It may go down to the mouth and be nevertheless finally rejected, or it may go only half way down before its direction is reversed. And further, the particle may travel back and forth many times in the funnel, or in the pouch, or through the extent of both, before either ingestion or rejection takes place. For the sake of shortening the description in the subsequent experiments this traveling back and forth of a particle will be described as the forming of "loops" in its path, each reversal from the ultimate direction (as determined by the fate of the particle) being considered as one loop. Such loops in the path of a particle in the funnel often take place while another particle in the pouch is either rejected or passed on into the funnel. These two sets of cilia may therefore beat quite independently of each other.

Other methods of rejecting or getting rid of substances are also frequently employed especially when there are large numbers of objectionable particles impinging on the Stentor's disk, as clouds of carmine or other indigestible material. In addition to a reversal of the membranellæ, bending away, contraction, and breaking of the foothold and swimming away, which reactions have been described by Jennings ('02) there are several other methods of reacting toward large quantities of indigestible particles which have not heretofore been described. Some Stentors close up the rim of the pouch almost completely for longer or shorter periods when surrounded by dense clouds of carmine. This method of preventing the ingestion of particles of carmine is most frequently observed after the Stentor has torn away from its foothold and is swimming freely in the water. This method is undoubtedly effective but for some reason the reaction is not persisted in for any length of time. Another and much more interesting modification of behavior occurs also under conditions similar to those which induce closure of the pouch, but it is observed in Stentors which have become attached again though still surrounded by dense clouds of carmine. Stentors under these circumstances are not quite as fully extended as when carmine is absent, but all the groups of cilia function as usual except with this interesting modification at times. The discal cilia instead of carrying the particles on toward the pouch as they strike the disk, roll the particles

around on the aboral side of the disk, in "push ball" fashion, in a rather large circle in the direction from right to left along the dorsal edge. (See Fig. 2.) Since the particles are not got rid of as soon as they strike the disk they accumulate in large, loose

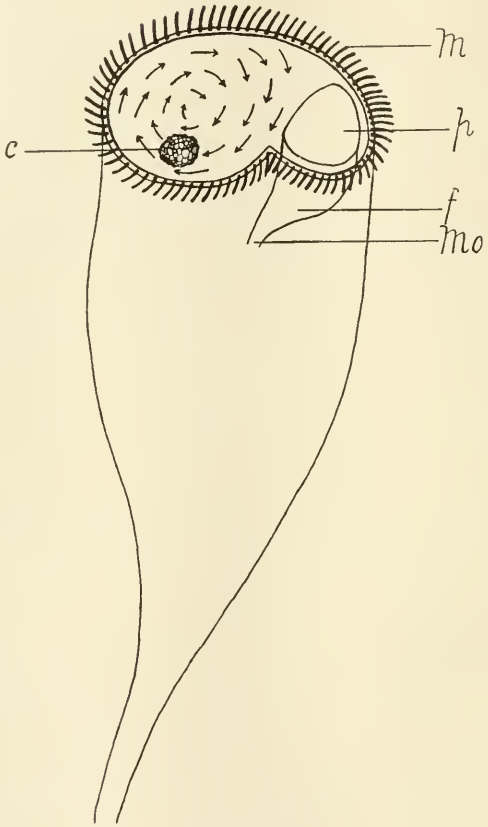

Fig. 2. Illustrating the "push ball" method of getting rid of objectionable particles. The arrows show the direction of beat of the discal cilia. *c*, mass of carmine grains; *f*, funnel: *m*, membranellæ; *mo.*, mouth; *p*, pouch.

masses which are, after some time, either by a special reaction or by accident—I didn't determine which—dropped over the edge of the disk, opposite the pouch. The carmine masses move slowly and it can be readily seen that the motion is altogether due to the action of the discal cilia. This method of preventing the ingestion

of indigestible particles is very effective. Very few particles ever get into the pouch so long as this mode of ciliary action takes place. This modification of behavior appears gradually and also disappears gradually; and during the transitions the action is imperfect, some particles passing into the pouch while others are carried to the right before dropping into the pouch. This phenomenon is interesting in that the discal cilia, which act usually in a certain definite coördinated way, are able to change their behavior so as to act in an entirely different but still coördinated way. What is more remarkable still perhaps is that some of the cilia beat in the same direction in both cases, while some others beat in an exactly opposite direction, the rest of them beating in every conceivable direction between their usual direction of beat and its direct opposite. This is a very good example of the extreme plasticity of behavior of such an organism as Stentor.

Under the same conditions in which the foregoing change of behavior was observed there was found another method by means of which the Stentor made its ingesting apparatus ineffective. This was done by contracting and staying contracted. Contractions for more than several minutes have not heretofore been recorded, but in one set of Stentors I observed continuous contraction for more than two and three-quarter hours. There was not a single relaxation during all this time and it is possible that this state of continuous contraction lasted longer than two and three-quarter hours, for the observation was not continued until relaxation occurred. The body cilia remained constantly reversed in this case, but the membranellæ frequently alternated between the usual and the reversed beat. Both groups of these cilia beat less vigorously than when relaxed or free swimming. The pouch and funnel were closed and no particles whatever were ingested.

Still other Stentors under similar circumstances differed in behavior from all the above. Under stimulation of dense clouds of carmine some Stentors swam with the foot ahead continuously for over three hours. The membranellæ were sometimes beat in the ordinary and sometimes in the reversed way, but always with a less vigorous beat than when the Stentor is fully extended attached, and in water with few particles present. There was no

spiral turning or revolving on the long axis. The Stentors swam in a circle as one would expect from a consideration of the crescentic shape of the partly extended Stentor. This method of behavior also resulted in the ingestion of but very few particles.

These are probably the chief constituents of what may be called the normal behavior of Stentor. We shall next consider the experiments which were designed to answer the question: Can Stentor discriminate between food and indigestible particles?

EXPERIMENTS WITH SPECIFIC PARTICLES

We have just seen that the normal behavior of Stentor is very complex for an organism of such simple anatomy, especially as far as the movements and action of the cilia are concerned. We saw that there are at least four distinct groups of cilia—five, if the pouch and funnel cilia are considered as two groups—each of which has a more or less definite thing to do under ordinary circumstances; but the moment that conditions obtain which are unusual, the behavior of one or more of these systems of cilia changes. We saw that any one of these four or five groups of cilia can change its behavior while the rest of the ciliary apparatus beat in the usual manner; or all the cilia of the entire Stentor can change direction and force of beat upon occasion so that entirely changed behavior results. In fact with these four or five groups of cilia which may beat independently of each other and vary their behavior in different ways, it seems hardly possible that a situation could confront a Stentor which could not satisfactorily be met by having recourse to the many possibilities of the varying behavior of these ciliary systems. The next problem then was to devise an experiment that should test the efficiency of this highly adaptive ciliary apparatus in discriminating between food and indigestible particles.

Experiment I. Discrimination between Phacus and Sulphur

For this purpose roll sulphur was ground up into a fine powder and then thoroughly stirred with a large quantity of water. After

the coarser particles had settled down the finer particles were siphoned off to be used in the experiment. The water in which the sulphur was stirred up was filtered water from the Stentor culture, so that the results of the experiment cannot be attributed to any peculiar qualities of the water. Some of the particles of sulphur were sucked up into a pipette together with some living Phacus triqueter, as previously described. This mixture of Phacus and sulphur was then fed on to the disk of a normally behaving Stentor in the fully extended condition, and the path and fate of each particle recorded. The results follow:

The particles are numbered in the order in which they reached the disk of the Stentor. The sulphur particles are denominated "s," the Phacus "p." Thus, 7s in the "rejected" column signifies that the seventh particle was sulphur, and that it was rejected. Where several numbers are bracketed, it signifies that these particles were fed simultaneously. In the column headed "loops" is shown the number of loops made by the particle. (See p. 11). The column headed "size" gives the size of the sulphur particles in units of the size of Phacus. Thus I, means same size as Phacus, .5 means one-half that size.

TABLE I

Experiment 1. Discrimination between Phacus and Sulphur

PARTICLES EATEN	PARTICLES REJECTED	LOOPS	SIZE	PARTICLES EATEN	PARTICLES REJECTED	LOOPS	SIZE
					15p		
1p					16p		
2p					17p		
3p				18p		2	
4p				19p		2	
5p				20p			
	6s		1	21p			
	7s		1	22p			
	8s	2	1	23p			
	9s	2	1	24p			
	10s	3	1		25s		1
11s		2	1		26s	3	·5
	12s		1		27s	3	·5
	13s		1		28s	3	·5
	14s		1		29s	3	75

SUMMARY

Eaten, 12 Phacus and 1 grain of sulphur.
Rejected, 3 Phacus and 13 grains of sulphur.

In the above experiment there were eaten 12 Phacus and 1 grain of sulphur, while 13 grains of sulphur and 3 Phacus were rejected. It is evident therefore that in this case there is some sort of discrimination between Phacus and sulphur.

Experiment 2. Discrimination between Starch Grains and Phacus

In another experiment designed for the same purpose but in which iodine-stained potato starch grains and Phacus triqueter were used, even more sharply defined results were obtained. The technique was similar to that of the preceding experiment, except that two pipettes were used, one for starch and the other for Phacus. The starch was stained with iodine to facilitate observation. Previous to feeding, the starch was washed very thoroughly to remove all the superfluous iodine. The results are as shown in Table II.

This experiment shows conclusively that Stentor can and does discriminate between two kinds of particles differing as much as Phacus and starch grains do from each other. The possibility of coincidence is entirely ruled out of court in that the stream of particles was changed at least seven times from starch to Phacus and from Phacus to starch. In the whole experiment there are only four "mistakes" at the most, including the rejection of a swarmspore twice and of a Coscinodiscus. But later experiments will show that the rejection of the swarmspore and Coscinodiscus were probably not mistakes, so that there occurred in this test only one mistake, that of ingesting particle numbered 69—a starch grain. The size of the starch grains was variable, being from one-eighth to four times the size of a Phacus specimen. This shows that in this experiment, as in the preceding, size was not the determining factor in the selection. Another point worth noticing is that there are no loops in the paths of the particles swallowed, and that there are very few particles rejected without loops. The 45 loops of particle 32 are probably due to the fact that the Stentor was at that time lying on its side with pouch uppermost, thus making the removal of the particle more difficult than usual. When the animal turned over the particle was gotten rid of. The usual number of loops does not exceed 10 in any one case.

s = starch grains; p = Phacus; c = Coscinodiscus; sp = swarmspore. Where (—) occurs it signifies that the group in which it is found was broken; that is, some of the members of the group were eaten while the others were rejected. Thus, in group 36p, 37p, 38p, (—), which consisted of four particles fed simultaneously, the first three were eaten while the last one was rejected. Size of starch is given in terms of Phacus.

TABLE II

Experiment 2. Discrimination between Starch Grains and Phacus

EATEN	REJECTED	LOOPS	SIZE	EATEN	REJECTED	LOOPS	SIZE
1p				36p			
2p				37p			
3p				38p			
4p				(—)	39c		
5p				40p			
6p				41p			
7p					42s	2	4
8p					43s	3	.125
9p					44s	2	.5
10p					45s	2	.5
11p				46p			
12p				47p			
13p				48p			
	14s		1	49p			
	15s		1	50p			
	16s		1	51p			
	17s	3	1	52p			
	18s	6	1	53p			
	19s	3	1	54p			
20p				55p			
21p				56p			
22p				57p			
23p				58p			
24p				59p			
25p				60p			
26p				61p			
27p				62p			
	28s	8	1	63p			
	29s	8	1	64p			
	30s	3	.5	65p			
	31s	2	.5	66p			
	32s	45	1	67p			
	33sp	2			68s	3	1
34p				69s			.5
35p					70s	3	1
30 minute intermission					71s	3	1

SUMMARY

Eaten, 50 Phacus, and 1 starch grain.

Rejected: 18 starch grains, 1 Coscinodiscus, and 1 swarmspore.

To determine what would be the effect of feeding in a mixed stream three kinds of particles, two that were not food and one that was food, natural starch grains, powdered glass, and Phacus triqueter were sucked up into different capillary pipettes and fed to a normal Stentor with the following results.

Experiment 3. Discrimination between Starch Glass, and Phacus

p = Phacus; s = starch grains; g = particles of glass. The particles of starch and glass were selected of a size about equal to that of Phacus.

TABLE III

Experiment 3. Discrimination between Starch, Glass and Phacus

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
	1s			18s	38	35p		
	2s			19s	38		36p	11
	3s			20s	38	37p		
	4s		21p			38p		
	5s		22p			39p		
	6s		23p			40p		
7s			24p			41p		
8p			25p				42g	
9p				26p	3		43g	
10p				27p	8		44g	
11p				28p	2		45g	
12p				29p	4		46g	
13p				30p	3		47g	
14p				31p	3		48g	
	15s		32p				49g	
	16s		33p		4		50g	
	17s		34p		2		51g	
							52g	

SUMMARY

Eaten, 21 Phacus and 1 starch grain.
Rejected, 12 starch grains, 11 particles of glass, and 7 Phacus.

At this point the Stentor contracted and swam away. The discrimination in this experiment is of about the same degree of accuracy as in the two preceding experiments. The specimens of Phacus which were rejected may not represent a mistake in discrimination at all, but may be due to a condition of partial satiety,

which will be taken up a little later. If this is the case the Stentor discriminated very well indeed what was Phacus and what was glass or starch. An interesting feature of this experiment is the number of loops that are recorded for the Phacus which were rejected and the absence of loops in the paths of the particles of glass.

So far, these experiments show that Stentor can select Phacus from a stream of mixed particles in which there are one or two kinds of indigestible particles mixed with Phacus. There are two possibilities as to the way this selection is accomplished. First it may be that Stentor ingests from a mixed stream only one kind of food particles (such as Phacus triqueter), and rejects all other kinds of food and indigestible substances. The other possibility is that Stentor ingests all sorts of food particles and rejects all sorts of particles that are not food. To determine which of these alternatives is the one which actually obtains, the following experiment was performed in which two kinds of food particles, Phacus triqueter and Euglena viridis, were fed in a mixed stream with two kinds of indigestible substances, powdered sulphur and powdered glass. The following are the results:

Experiment 4. Selection of Phacus and Euglena from Sulphur and Glass

The Stentor upon which this experiment was tried was the same one which had submitted to the second experiment, on the previous day (p. 90). It still contained considerable amounts of partly digested food which probably represented the Phacus that were eaten the day previous. The starch grain which was eaten in Experiment 2 was not to be seen, and it is probable that it was voided some hours after it was ingested. That it was digested is made highly improbable in view of the work of Meissner ('88) who found that nearly all of the potato starch which the Stentors ate was not digested, and that some of the starch which remained in Stentors for over 48 hours was practically in the same condition as when fed. There is therefore no good ground for supposing that any part of the behavior of the Stentor in this experiment was due to the fact that a starch grain was ingested on the

previous day. As later experiments will demonstrate, however, the ingestion on the day before of the 50 Phacus has probably influenced the behavior of Stentor, and it is pretty certain that the condition of partial satiety which is exhibited in the beginning

p = Phacus; e = Euglena; s = sulphur; g = glass; t = encysted Trachelomonas volvocina.

TABLE IV

Experiment 4. Selection of Phacus and Euglenæ from Sulphur and Glass

EATEN	REJECTED	LOOPS	SIZE	EATEN	REJECTED	LOOPS	SIZE
	1p	5			21g	3	1
	2p	3			22g	3	2
	3p	2			23g	2	.5
4p					24g		1
5p					25g		1
	6p				26s		.25
7p					27s	2	25
8p					28s	2	1
9p					29s	3	1
10p					30s	5	.5
11p					31s	5	.5
12p				32e			
13p				33e			
(-)	14p			34e			
(-)	15p				35t		
(-)	16p			36e			
(-)	17p			37p			
18p				38p			
19p				39p			
	20g	3	1	40p			

SUMMARY

Eaten, 15 Phacus and 4 Euglenæ.

Rejected, 8 Phacus, 6 particles of sulphur, 6 particles of glass, and 1 Trachelomonas.

of Experiment 4 was due to this cause. I think it will be pretty clearly shown that the rejection of the first three Phacus in Experiment 4 was not a "mistake" on the Stentor's part but probably represents a transition from a condition of partial satiety to one of hunger, brought about by the stimuli of the Phacus upon the pouch and funnel. The Phacus numbered 14, 15, 16, 17—the last four of a group of nine—were rejected probably because of the

inconvenience or impossibility of swallowing as many as nine Phacus at once. In very hungry Stentors six are sometimes swallowed at one gulp, and once a Stentor was observed to swallow seven, but in groups of more than seven some were always rejected.

So then in this experiment there are probably no mistakes in discrimination whatever unless the rejection of a Trachelomonas volvocina is considered a mistake. But the Trachelomonas was in the resting stage and inactive, as were also the swarmspore and the Coscinodiscus in Experiment 2. There is a possibility therefore that the inactive particles are rejected and that only moving, active particles (organisms), are ingested. To determine whether selection is made upon this basis, some Euglena viridis were taken from a culture and killed in various ways, by heat, alcohol, acetic acid, etc. They were then thoroughly washed and sucked up into capillary pipettes, those killed by heat in one pipette, those killed by alcohol in another, and so on. Another pipette was then filled with normal living Euglenæ. Normal Stentors were then isolated and fed with these Euglenæ in various conditions in a mixed stream, precisely as was done with the various substances in the preceding experiments. There was no discrimination observed between any of the differently prepared Euglenæ. The dead Euglenæ were eaten with the same readiness as were the living. Some Stentors rejected about equal numbers of each, while others rejected all of both kinds. Similar experiments were tried using Phacus and Trachelomonas as food, with the same results. There was no selection between the living and the dead organisms.

Another possible way of explaining how the rejection of the Trachelomonas, Coscinodiscus, and the swarmspore was made, is that Stentor may be able to distinguish the different kinds of food particles from each other, and that certain kinds of food may be eaten with more readiness than others. This kind of selection may, perhaps, take place under all conditions, or only under certain conditions. It may depend on the relative number of the different kinds of particles, or the order in which they come. To determine this matter a number of experiments were designed.

The first experiment was designed to show whether selection was exhibited when Phacus triqueter and Phacus longicaudus were fed in mixed order, Phacus triqueter being much more numerous than Phacus longicaudus.

Experiment 5. Discrimination between Phacus triqueter and P. longicaudus

(See Table V)

Out of the five Phacus longicaudus which were fed with the 176 Phacus triqueter, four were rejected and one was ingested. The four rejected ones were the last members of the respective groups in which they were fed, and the one which was ingested cane first in the group of two—*1661*, 167. It was not positively ascertained in any of my experiments whether discrimination is nicer among the last members of a group than among the first, but the evidence seems to point that way. But even if this should turn out not to be true, it seems clear that actual discrimination between Phacus triqueter and P. longicaudus took place in this experiment.

Experiment 6. Discrimination between Different Species of Organisms

In another experiment designed to further show selection of one or more kinds of food particles from as many as six different species of organisms, the following results were obtained. There were fed in mixed order, Euglena viridis, Euglena deses, Phacus triqueter, Phacus longicaudus, Trachelomonas hispida, and Trachelomonas volvocina. All these organisms were fed from a single pipette on to the disk of a Stentor as in the preceding experiment. For results see Table VI, p. 99.

Experiment 7. Discrimination between Different Species of Organisms

Immediately following the above experiment I fed another Stentor with the same sorts of flagellates (but omitting the Euglena deses which is difficult to handle in a capillary pipette owing to its habit of sticking to the walls) with the results shown in Table VI, p. 100. Loops are not recorded.

The numbers followed by *l* are Phacus longicaudus; all the other numbers represent Phacus triqueter.

TABLE V

Experiment 5. Discrimination between Phacus triqueter and Phacus longicaudus

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1			45			89		
2			46			90		
3			(-)	47	7	91		
4			(-)	48	4	92		
5			49			93		
6			50			94		
7			51			95		
8			52				96	5
9			53			97		
10			54				98	4
11			55				99	3
12			(-)	56	6		100	3
13			57				101	
14			58			102		
15			(-)	59	21	103		
16				60	3	104		
17			61			105		
18			62			106		
19			63			107		
20			64			108		
21			65			109		
22			66			110		
23			67			111		
24			68			112		
25			69			(-)	*113l*	2
26			70				114	6
27			71				115	6
28			(-)	72	4		116	5
29			(-)	73	3		117	4
30				74	3		118	8
31				75	2		*119l*	3
32			76			120		
33			77			121		
34			78			122		
35			79			123		
36			80			124		
37				81	2	125		
38			82				126	4
39			(-)	83	3	127		
40				84	4		128	11
(-)	41	4		85	2	129		
42				86	5	130		
43				87	3	131		
	44	3		88	3	(-)	132	1

TABLE V—continued

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
133			149			*166l*		
(-)	134	3	(-)	150	6	167		
	135	1	(-)	151	4	168		
136			152			(-)	169	
137				153	4	170		
138				154	4	171		
139			155			(-)	172	4
140			156			(-)	173	4
141				157	1	(-)	174	1
142				158	1		175	2
143				159	8		176	2
144			160			177	*179l*	1
145			161			178		
146			162			(-)	*179l*	1
147			163				180	6
148			164					
			(-)	*165l*	1			

At first sight neither Experiment 6 or 7 seems to show that Stentor eats some kinds of food with more readiness than other kinds, for some individuals of each of the various kinds of flagellates were eaten while some of every kind were rejected. But upon closer examination it is found that the latter parts of the experiments are different from the earlier in several respects. A larger proportion are rejected at the end of each of the experiments than at the beginning. Of the organisms eaten the variety is much more extensive at the beginning than in the latter part of the experiments. All the organisms that were fed in the beginning of both experiments were ingested, but at the close only Euglena viridis and Trachelomonas volvocina were ingested, all the other kinds being rejected. Only a single Trachelomonas hispida was ingested. It is clear that there occurred a change in the physiologic state of Stentor as each of the experiments progressed. In the sixth experiment, of the six different kinds of flagellates that were fed, Euglena viridis and Trachelomonas volvocina were eaten with the greatest readiness. After the twenty-second organism had been fed, ten Euglenæ viridis were ingested and five were rejected. The fact that not all the Euglenæ were

ev = Euglena viridis; ed = Euglena deses; pt = Phacus triquetur; pl = Phacus longicaudus; th = Trachelomonas hispida; tv = Trachelomonas volvocina.

TABLE VI

Experiment 6. Discrimination between Different Species of Organisms

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1pt			30ev				59pl	
2ed			(-)	31ev			60ed	
3ev				32pl		61ed	62pl	
4ev				33ev				
5ev				34pl			63pl	
6ev				35pl			64ch*	5
7ev				36pl			65tv	2
8pl				37pl			66tv	2
9ev				38pl			67pl	
10ev				39pl			68pl	4
11pl				40pl	5			
12pl			41ev			Three minute intermission. Pipette was refilled with food or-organisms.		
13pl			42tv					
14pl				43th				
15pl				44ev				
	16ev			45th			69ev	
	17pl			46th			70ev	
	18pl	2		47ev	4		71ev	
19ev				48pl			72ed	
20pt			49tv				73ev	
21pl			50ev				74ev	
	22pl	2	51ev				75ev	
23ev				52pl			76ev	
24ev				53th			77ev	
25ev			54ev				78ev	
(-)	26ev		55ev				79ev	
	27pl			56th			80ev	
	28pl		57ev				81ev	
	29pl			58th				

*Food particle 64 was a Coleps hirtus.

ingested does not prove that there was no selection going on in this part of the experiment, for the Phacus triqueter and the Trachelomonas hispida are without exception rejected. The explanation of this apparently capricious selection of some Euglenæ and the rejection of the rest is probably to be sought in the changed

condition of the Stentor brought about by the particles which were just previously ingested. That is, the Stentor was probably in a condition of partial satiety, where less and less food is taken even if it should meet all the requirements of a perfect food under conditions of hunger. The actual proofs of such conditions of partial and complete satiety will be taken up later on, but it may be pointed out here that in the sixth experiment the Stentor apparently grew less and less hungry as the number of ingested particles increased.

Particles designated as in Experiment 6

TABLE VII

Experiment 7. Discrimination between Different Species of Organisms

EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED
1pl		15pl		30ev		45tv	
2pl		16pl			31pl	46tv	
3pl		(–)	17pl		32pl		47th
4pt		(–)	18pl	33ev		48tv	
5pl		19pl		34pl			49pl
6pl		20pl		35ev		50tv	
7pl		21ev		36pl			51pt
8th		22pl		37ev			52pl
9ev		23ev		38ev		53tv	
10pt		(–)	24pl		39tv		54ev
11pl		25ev			40th		55th
	12pl	26ev			41pl		56th
13pl		27ev			42pl	57tv	
14pl		28pt			43pl		58ev
		29pl		44tv			59ev

The proportion of Euglena viridis and Trachelomonas volvocina was not the same in the two experiments, so no conclusions may be drawn with regard to which of these two kinds of organisms is eaten with the greater readiness. But it is of course clear that Euglena viridis and Trachelomonas volvocina are "preferred" by the Stentor to Phacus triqueter, P. longicaudus, and Trachelomonas hispida; which is only another way of saying that Stentor expresses a choice in the food which it eats.

Experiment 8. Discrimination between Organisms of Similar Size and Shape

As a final test in food discrimination in which the path and fate of each particle was recorded the following experiment was designed and performed. Two kinds of organisms were used, one being Trachelomonas volvocina and the other a species of Euglena which upon very slight disturbance contracted into a spherical mass of almost exactly the same size as that of a Trachelomonas. The organisms were fed from a capillary pipette in a mixed stream, the number of Euglenæ and Trachelomonas being as nearly equal as possible. The loops were not recorded. The results follow:

e = Euglena; t = Trachelomonas; th = Trachelomonas hispida

TABLE VIII

Experiment 8. Discrimination between Organisms of Similar Size and Shape

EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED
1e			16t		32e		47e
2e			17t		33t		48e
3e		18t			34t	49e	
	4e		19t		35e		50t
5t			20t		36e		51t
6t			21t	37e			52t
7e		22e			38t		53t
8e		(-)	23e		39t		54e
9e			24t	40e			55t
	10th		25t		41t		56t
	11e		26e		42e		57e
	12e		27e		43t	58e	
	13t	28e		44e			59t
	14t	29e			45t	60e	
	15t	30e			46t		61t
		31e					62e

Of the 62 organisms 32 were Euglenæ, 29 Tracheolmonas volvocina, and 1 T. hispida. Seventeen Euglenæ were eaten and 15 rejected; and of the Trachelomonas 3 were eaten and 27 rejected. That the Euglenæ were eaten with more readiness than the Trachelomonas is evident from an inspectoin of these figures, which

show that 56 per cent of the Euglenæ were eaten and only 10 per cent of the Trachelomonas. But a better and truer idea of the accuracy of selection is obtained by a study of the results as set forth in the table. As in the sixth and the seventh experiments, the beginning of this experiment differs from the latter part in that the variety of ingested particles is greater in the beginning, and also in that the proportion of food ingested as compared with the amount fed, is greater in the beginning. There seems also to be a marked change in the basis upon which discrimination is effected; it evidently became more restrictive towards the end.

What is the cause of the change in the degree of restrictiveness in selection in these experiments? Is the change due to differences in the food or to an alteration in the Stentor itself?

Evidently different food particles possess different strengths or powers of giving the stimulus that causes Stentor to ingest them. The stimulus from Phacus triqueter is stronger than that from Trachelomonas hispida; and that from Euglena viridis is still stronger than that from Phacus triqueter. This is shown by the fact that at certain times (when nearly replete), Stentor takes only Euglenæ (or Phacus if Euglenæ are not present), from a mixed stream of organisms. There probably are slight differences between the strengths of the stimuli from different individuals of the same species, but these differences are evidently less than those between different species, for when nearly satiated, Stentor takes only the particles of one species.

Yet we find in the experiments described above that specimens of the same organism (as Euglena) are at first eaten without any exception, while later less than half of them are eaten. The change in the proportions eaten as the experiment progresses are well seen in the following tabulation of the results of the eighth experiment. The experiment is divided into successive groups of about ten particles to each group.

TABLE VIII*a*

Tabulation of Results of Experiment 8

GROUPS	EUGLENAE EATEN		EUGLENAE REJECTED		TRACHELOMONAS EATEN		TRACHELOMONAS REJECTED	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1–10	6	86	1	14	2	$66\frac{2}{3}$	1	$33\frac{1}{3}$
11–21	0	0	2	100	1	11	8	89
22–30	4	57	3	43	0	0	2	100
31–40	3	50	3	50	0	0	4	100
41–50	2	40	3	60	0	0	5	100
51–62	2	40	3	60	0	0	7	100

As this table shows, the percentage of Euglena eaten decreases steadily throughout the experiment, from 86 per cent to 40 per cent; while that for Trachelomonas decreases from $66\frac{2}{3}$ per cent to 0. The percentage of Euglena rejected increases from 14 per cent to 60 per cent; of Trachelomonas from 33 per cent to 100 per cent. Similar changes were found in all the experiments of like character with Stentor, including a number which I do not publish in detail. It is evident that this regular change cannot be due to chance variations in the food, but must be due to an alteration in the physiologic state of Stentor itself. In some way the Stentor changes as it takes food, so that stronger and stronger stimuli are required to set off the ingesting mechanism. Such a change is of course parallel with what we observe in higher organisms.

We find therefore that we have in this unicellular organism physiologic conditions corresponding to what we call hunger and satiety in higher forms. Differences in behavior due to hunger and satiety have not heretofore been demonstrated for those protozoa that secure their food by means of an alimentary vortex. The experiments we have just described, primarily designed to test the power of selection, indicate strongly the existence of such differences. Let us now turn to experiments that were planned to test this matter. We wish to determine what differences exist between hungry and satiated Stentors, and whether there are intermediate conditions, manifesting themselves in differences in behavior.

Experiment 9. Effect of Hunger on Behavior in Feeding

Eight Stentors with some of their own culture solution were placed into a small preparation dish. After they had attached themselves I selected a large individual and fed it with Phacus triqueter with the result shown in Table IX.

Experiment 10. *Effect of Satiety on Behavior in Feeding*

At the close of the foregoing experiment the dish of Stentors was set aside until next day when it was found that the Stentor which was fed in this experiment contained a considerable number of small brownish bodies. These upon close examination were found to be Phacus triqueter in a stage of partial digestion. The protoplasm was extracted from the Phacus and the chlorophyll was changed in color. Here was then a good opportunity to see whether the ingestion of Phacus on the previous day had any effect upon the ingestion of food particles today. A mixture of Euglena viridis and Phacus triqueter was fed from a capillary pipette on to the disk of Stentor with the results shown in Table X.

Perhaps the most notable difference between these two experiments is in the number of food particles ingested. In the ninth experiment 73 out of 118 particles were eaten, the majority being ingested in the first half of the experiment, while in the tenth only 10 were eaten out of 75, and these ten were distributed comparatively evenly. A large number of experiments performed upon well-fed Stentors showed substantially the same results as Experiment 10. In no case where the membranellæ and pouch cilia beat normally, i e., where their movement was not reversed, did it happen that every single particle was rejected. Some few were always ingested if the experiment was sufficiently extensive. The lowest ratio of particles eaten to those fed was 1 to 12. When the particles were fed very rapidly the ratio was very much increased. But if the particles are fed at the rate of about 100 an hour, results like those of Experiment 10 will be obtained. Stentors from artificial cultures in which food is very plentiful, and in which the Stentors thrive and reproduce very rapidly, show substantially the same results as are shown in Experiment 10.

TABLE IX

Experiment 9. Effect of Hunger on Behavior in Feeding

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1				47	1		87	2
2				48	2		88	4
3				49	3	89		
4			Stentor contracted; hit with the pipette.			(-)	90	2
5						(-)	91	3
6						92		
7				50	2		93	1
8				51	2		94	1
9				52	2		95	1
10		3		53	1		96	1
11				54	1		97	1
12				55	1	98		
13				56	5	99		
14			57			100		
15			58			(-)	101	1
16			59			(-)	102	2
17		6	60				103	1
18		4	61				104	1
19			62				105	1
20			63				106	1
21			64				107	6
22			(-)	65	2	108		
23			(-)	66	2		109	7
24			67					
25			68			At this point a Stylonychia was fed twice to the Stentor but was rejected each time. On being fed the 3d time the Stentor held the S. in the pouch for an instant and then contracted, setting the S. free. The S. was fed again to the Stentor and was eaten. The Stentor then contracted for a few minutes.		
26		4	69					
(-)	27	7	70					
28			71					
29			72					
30			73					
(-)	31	5	74					
(-)	32	5	75		1			
33			(-)	76	4			
34			77					
35			78					
36			79				110	1
37			(-)	80	2		111	1
38			81		6		112	1
(-)	39	3	82				113	1
40			83		2		114	1
(-)	41	1				115		
42			Contracted; hit with the pipette.			(-)	116	2
43						117		
(-)	44	1	84			(-)	118	1
(-)	45	3	85					
46			(-)	86	2			

e = Euglena viridis; p = Phacus triqueter

TABLE X

Experiment 10. Effect of Satiety on Behavior in Feeding

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
	1e	1		44e	1
	2p	1		45e	1
	3p	1		46e	1
	4e	1		47e	1
	5e	1		48e	2
	6e	1	48 fed again	(49e)	3
	7e	1	50e		
	8e	1		51p	1
	9e	1		52p	1
	10e	1		53p	1
	11p	4		54p	1
	12e	2		55p	1
13e				56p	1
14e				57p	1
	15e	1		58p	1
16e				59e	3
(-)	17e.	1		Bit of Debris	1
	18e.	1	Fed again	(Bit of Debris)	1
18 fed again	(19e)	1		60p	1
18 fed again	(20e)	1		61p	1
18 fed again	(21e)	1		62p	1
18 fed again	(22e)	1		63p	1
18 fed again	(23e)	1		Bit of Debris	1
18 fed again	(24e)	1		64p	1
18 fed again	(25e)	1		65p	1
18 fed again	(26e)	3		66p	1
18 fed again	(27e)	2		67p	2
18 fed again	(28e)	1		68p	3
18 fed again	(29e)	1			
	30e	1	At this point the Stentor contracted. When again expanded, Stentor reversed the cilia when the stream from the pipette reached the disk. After about a minute the cilia beat normally.		
31p					
(-)	32e	1			
33e					
(-)	34e	1			
	35e	1		69e	2
	36e	2		70e	1
	37e	1	71e		
	38e	1	72e		
	39e	1	(-)	73e	3
	40e	2	(-)	74e	1
	41e	1		75e	1
42e					
43e					

Perhaps the most significant difference of all between the ninth and the tenth experiment is with regard to the occurrence of loops in the paths of the organisms which were fed. These two experiments are typical in this respect of nearly all the experiments which form the basis for this paper.

We find, first, that nearly all the loops occur in the paths of the rejected particles. As it happens in these two experiments, no particle is rejected without at least one loop in its path. Second, when loops occur in the paths of ingested particles they are generally found only in those experiments where a comparatively large proportion of the particles are ingested, or in other words, when the Stentor is hungry or only in the first stages of satiety. Third, in extensive experiments like the two preceding, more loops occur in the first half of the experiments than in the last half, both in the ingested and in the rejected particles. Fourth, very few loops occur in feeding hungry Stentors. Fifth, very few loops occur in a satiated Stentor. Sixth, the maximum number of loops is found when the Stentor is in the first stages of satiety. Seventh, in a stream of mixed particles including food and bits of glass or sand, the glass or sand is generally rejected with fewer loops than the food particles. A conspicuous case of this is seen in the third experiment.

What is the real significance of these loops? Are loops the result of fatigue of the ingesting or of the rejecting mechanism, or are they correlated with a certain physiologic state of Stentor in such a way as to be a factor in the selection of food? It appears clear that the loops are not due to fatigue of the ingesting or rejecting mechanisms, nor of the apparatus for receiving the stimuli. Fewer loops are made when the Stentor is satiated than when only partial satiety sets in; this would not be the case if the occurrence of loops were due to fatigue, as will presently appear. Further, when excessively small particles are fed, such as the Euglenæ in the eighth experiment, as many as 11,000 may be eaten and several times that number rejected, all within two or three hours, so that fatigue could hardly occur from handling a few hundred as in Experiments 9 and 10. Again we have seen that discrimination is more precise as the Stentor becomes satiated. This shows that

the apparatus for receiving stimuli has not become effectively fatigued.

Thus it appears clear that the occurrence of loops is not due to fatigue; we must look for the explanation in some other change in the physiologic state of the animal. As noted above, the loops appear as the animal approaches satiety, so that there can be little doubt but that the change in the degree of hunger is what brings on the loops. The loops, as we have seen, almost always occur in the paths of particles that are finally rejected. It appears that the rejecting mechanism is not set in operation by the first slight stimulus from an objectionable particle, but the stimulus seems to be summated with every successive loop that is made, until it is finally strong enough to cause rejection. In the first stages when the animal is hungry the ingesting mechanism is more readily set off than that for rejection; near repletion, the rejection apparatus is more readily set off; and as repletion advances the rejecting apparatus is continually more and more readily set off, requiring therefore fewer loops. The apparatus for receiving stimuli seems therefore to be in a state of continual change, so that stimuli which readily set off the ingesting reaction when the animal is hungry have but a slight effect when the Stentor is nearly replete, and finally have no effect at all, or set off only the rejecting reaction.

A difference is seen in the fact that when a particle is accepted, this is done (as a rule) at once, without loops, while rejection is done only after some delay, with the occurrence of loops. Only when the Stentor is fully satiated does rejection occur instantaneously. In the state of incomplete satiety, rejection is a slow and uncertain process, as if the stimulus for rejection had first to gather strength before rejection could occur.

Thus the number of loops which occur before rejection depends on the degree of hunger. There is doubtless a similar though reverse effect of hunger on the ingesting apparatus; it seems certain that a stronger stimulus is required to set off the ingesting reaction when the Stentor is partially satiated than when very hungry. But the evidence is not so clear as for the rejecting apparatus owing to the fact that loops rarely occur in the path of

a particle that is to be ingested. The nature of the assumed rejecting and ingesting mechanism and the way selection is brought about, will be discussed later on.

We have thus far dealt mainly with what may be called stages of moderate hunger and moderate satiety. There still remain the states of utter hunger and of surfeit to be described.

The condition of extreme hunger may be produced by putting a number of Stentors with as little of their culture solution as possible into clear tap-water and keeping the water as free from bacteria as possible. There is no evident difference in behavior between Stentors in the condition of extreme hunger and those moderately hungry.

The condition of utter satiety is more interesting. The behavior of a Stentor in such a state is very different from the normal behavior. The following experiment fully illustrates this. Several hundred small paramecia were placed with a few Stentors in a watch glass for two days, so that the Stentors could feed upon them. The largest of the several Stentors was then selected for feeding with Trachelomonas hispida. The Stentor was filled with a number of globular masses of slightly brownish transparent material which probably represented as many broken down paramecia. The Trachelomonas were fed from a capillary pipette as usual. Following are the results.

Experiment 11. The Effect of Utter Satiety on Stentor

In this experiment the figures, 1 to 24, signify as many Trachelomonas hispida which were fed to Stentor. As in preceding experiments, they are numbered in the order in which they were fed. Stage "pipette presented," means that the end of the capillary pipette which contained Trachelomonas hispida was brought within about half a millimeter from the animal's disk, and that a very slow stream of water was then caused to flow against the disk. All the other stages of behavior are self-explanatory.

Pipette presented
1 Trachelomonas hispida rejected with 1 loop
Contraction
All cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction for 4 minutes
Cilia normally active
Pipette presented
Contraction
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pippette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active

Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active
Pipette presented
Slowing of cilia
(Pipette continued)
Bending away
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Bending away
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Bending away
Pipette removed
Cilia normally active
Put a Trachelomonas on the disk near the pouch with a very thin glass rod.
2 rejected, with 5 loops.
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Trachelomonas presented with glass rod.
3 rejected with 7 loops
Trachelomonas presented with glass rod
4 rejected with 4 loops
Pipette presented

Slowing of cilia
Pipette removed
Cilia normally active
Trachelomonas presented with glass rod
5 rejected with 4 loops
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented Stream very slow
6 rejected with 3 loops
7 eaten
8 rejected with 4 loops
9 rejected with 8 loops
10 rejected with 4 loops
11 eaten
12 (Coscinodiscus) eaten
13 rejected with 8 loops
14 rejected with 4 loops
15 rejected with 8 loops
16 rejected with 5 loops
17 rejected with 3 loops
18 rejected with 3 loops
19 rejected with 9 loops
20 rejected with 3 loops
21 rejected with 4 loops
22 rejected with 5 loops
23 rejected with 7 loops
24 rejected with 2 loops

This Stentor was at no time as fully extended as the average Stentor is when not containing much food, nor was the ciliary action quite so strong and vigorous as when normally hungry. This experiment is a good example of a remarkable change in the physiologic state of Stentor in that the Stentor was slowly brought from a condition of surfeit to one of only partial satiety. The change was a gradual one inasmuch as five Trachelomonas impinged upon the disk before the change was complete. A single Trachelomonas caused apparently no visible change in behavior. There seems to have been required the summated stimuli from five Trachelomonas before the state of surfeit could be changed to one of only partial satiety.

There was also a gradual decrease in irritability arising from the stream of water from the pipette. At first this caused contraction. A little later only a slowing of cilia occurred—the initial stage of contraction. Still later the Stentor bent away from the source of the stimulus, and finally the stream from the pipette no longer caused any visible reaction.

This decrease in irritability is parallel with the decrease in satiety but is probably not due to the same cause. The cause of the decrease was probably the frequent repetition of the stimulus, the faint stream of water from the pipette, for a marked decrease in irritability resulted before the glass rod was used. But the

change from a condition of surfeit to one of only partial satiety was caused by the impinging of five Trachelomonas on the Stentor's disk.

This decrease in the condition of satiety does not represent a similar rate of decrease of food in the Stentor's body. We see therefore that a particular state of hunger in a Stentor does not directly nor necessarily accurately represent the amount of food in the Stentor at the given moment. What the state of hunger actually represents is the condition of the organ for receiving stimuli from external food particles. This is influenced: (1) by the past history of the amount and kind of stimulation from external particles; (2) by the amount of food in the Stentor's body.

Other peculiarities of behavior attendant upon the condition of satiety are the following:

1 Extension is always sub-maximal. Instead of being extended as fully as possible with the disk spread so as to present the greatest area to the base of the vortex set up by the membranellæ, the Stentor is only partially extended and the disk is smaller. The animal does not extend perpendicularly upward or horizontally from its base of attachment, but generally hangs downward, or frequently lies upon some debris, etc., if possible.

2 The aboral side is more strongly convex when replete than when hungry. This posture may be related in some way to the voiding of excrementa, though no evidence could be obtained to show that this is true.

3 There is a marked decrease in the activity of the membranellæ. This may have much to do with the degree of extension in Stentor. Strong action of the membranellæ tends to pull the disk away from the foot, and therefore full extension may be partly due to the strong beat of the membranellæ. If the membranellæ beat only in a weak manner there is no such pull upon the Stentor, and as a result it lies prone or hangs downward from its point of attachment. This is made still more probable by the fact that hungry, free-swimming Stentors are seldom as fully extended as attached ones.

4 Satiated Stentors are very irritable to stimuli affecting the swallowing or rejecting mechanisms. In hungry Stentors one

can poke around the pouch a good deal before a Stentor contracts, but in a satiated specimen the faintest touch generally causes contraction. But mechanical stimuli on the sides of the body do not seem to cause contractions more readily in replete than in hungry Stentors.

5 If the stimuli are not too strong, contraction is often resolved into stages, and only the first of these may be passed through, instead of all of them as is the case when the Stentor is hungry. Thus contraction may be resolved into the following separate stages. (*a*) Cessation of action of the membranellæ. (*b*) Closure of the pouch. (*c*) Gradual rounding up of the anterior and posterior portions of the Stentor into an oblong mass. If the stimulus still continues, complete contraction follows; but this act changes the form and size of the already partially contracted Stentor very little. No such slow contraction takes place in a hungry Stentor where, if the mechanical stimulus is strong enough to cause cessation of action of the membranellæ, the entire process of contraction occurs instantaneously.

EXPERIMENTS WITH MIXTURES OF PARTICLES

In this part will be considered experiments in which the food particles were not fed and observed individually, but in which the Stentors were surrounded by mixtures of particles of various kinds. The purpose was to determine whether the animals can make a selection from among the different particles of such a mixture. After remaining for various periods of time in such mixtures, the Stentors were placed on a slide and compressed with a cover glass. It was then possible to estimate with some accuracy the relative amounts of the various kinds of particles that had been ingested. For such experiments we can use only particles that remain long in suspension, so as to maintain their relative distribution. Heavy particles cannot be employed.

It is obvious that this method gives less precise results than employed in the foregoing experiments. But by its use certain additional problems can be attacked. We have seen that Stentor discriminates between different sorts of particles fed in

succession; can it also make a selection from among particles that are intimately mixed? And why does it at times take particles that are not good for food, such as carmine?

In the first series of experiments the following procedure was adopted. In each of several small preparation dishes there was placed 10 to 20 cc. of filtered fluid from the Stentor culture, together with about 30 Stentors that contained no solid food. Into some of the dishes was introduced a mixture consisting half of carmine particles, half of an admixture of Chlamydomonas with some very small Euglenæ. In others, serving as control carmine alone was introduced, in amount equivalent to the total quantity of particles in the other dishes.

The contents of the dishes were thoroughly stirred, and they were then placed in a dark box to prevent the Chlamydomonas from collecting at the lighted side of the dish. After half an hour the Stentors were examined under the microscope. The average content for each Stentor was about 1500 Chlamydomonas, about 85 Euglenæ, and carmine of the bulk of about 10 Euglenæ. Several Stentors had ingested about the same amount of food, but no carmine whatever.

About half the Stentors were left surrounded with these substances for 24 hours. These then contained a much greater amount of Euglenæ and Chlamydomonas than before, but in about the same proportions. But the carmine content was practically nil. This is probably explained by the fact, brought out in the first series of experiments, that discrimination becomes more perfect as hunger becomes less. Having become in the later hours nearly satisfied, the Stentors discriminated more accurately against the carmine, and meanwhile that which they had ingested in the first hours of the experiment had been egested in the natural course of events. The result was not due to the settling of the larger particles of carmine to the bottom, since when I added fresh carmine, none of it was ingested. It is also improbable that the Stentors had become "educated" to the fact that carmine is not food, as will be shown later.

In the control dishes where only carmine was present, the Stentors had ingested an amount of carmine equal to about two-thirds

of the quantity of substance taken in by the Stentors in the dishes containing both food and carmine. There was variation among different individuals in the amount taken, but none were entirely devoid of carmine.

A similar series of experiments in which india ink was substituted for carmine gave the same results, except that only about half as much india ink was ingested as carmine.

These experiments show that in an intimate mixture Stentor can discriminate and select with a high degree of accuracy such minute food particles as Chlamydomonas from among indigestible particles like carmine or india ink. In some cases the accuracy of selection is almost perfect. Further, it is seen that the larger amounts of carmine are eaten when no food is present, while little or none is ingested when food is present also.

In another experiment the Stentors came from a culture where food was more abundant. They were fed with mixtures containing one or more of the following: yeast, carmine, india ink, Euglenæ, Trachelomonas. Nine different combinations of these materials were made in separate dishes. Equal quantities of the different substances were employed in each experiment. At the end of an hour the Stentors of each dish were examined as to the materials ingested. The different combinations employed and the results obtained are given in the following series.

1 Mixture of yeast and carmine. Result: Stentors at the end of an hour contained about twice as much yeast as carmine. The total bulk ingested was equal to that of about 600 Euglenæ.

2 Yeast and india ink. Result: Less yeast ingested than in 1. Very little ink was eaten—about the bulk of three Euglenæ.

3 Euglenæ, Trachelomonas, and carmine. Result: Many Euglenæ and Trachelomonas ingested. Carmine to the bulk of about 15 Euglenæ.

4 Euglenæ, Trachelomonas, and ink. Result: Same quantity of Euglenæ and Trachelomonas a in 3. Ink to the bulk of 1 Euglena.

5 Yeast, Euglenæ, Trachelomonas, and carmine. Result: More Euglenæ and Trachelomonas then yeast. Carmine to the bulk of 9 Euglenæ.

6 Yeast, Euglenæ, Trachelomonas, and ink. Result: More Euglenæ and Trachelomonas than yeast. No ink.

7 Ink. Result: Ink to the bulk of 3 Euglenæ.

8 Carmine. Result: Carmine to the bulk of 20 Euglenæ.

10 Yeast, Euglenæ, and Trachelomonas. Result: More Euglenæ and Trachelomonas then yeast.

Only about half the Stentors of each dish were examined at the end of an hour. The others were allowed to remain in the dishes until next day. Examination then showed somewhat the same results as are described above. In dish 4 however, no ink was found in any of the Stentors, and in all the dishes in which ink and carmine were placed the amount of these substances ingested by the Stentors was less. The amount of yeast in the Stentors in dishes 6 and 5 was also considerably less than on the previous day. This decrease may have been due, in part at least, to the torulas sinking to the bottom, notwithstanding the frequent stirrings to which the dishes were subjected.

These experiments show that Stentor can discriminate between the torulas of yeast and the grains of ink or carmine, and that selection is more perfect for example, between yeast and ink than between yeast and carmine They show also that less ink and carmine are eaten when food is present than when not. But in the case of dish 1 the Stentors are found to contain more carmine when the yeast is also present than when only carmine is present as in dish 8. I am unable to explain why this is so in this particular case.

These two sets of experiments are probably sufficient to show that Stentor can discriminate as well when enormous numbers of particles touch the disk and pouch continuously for 24 hours, as when a small number are swept into the pouch in rather slow succession, and that very minute particles of different sorts can be sorted as accurately and rapidly as large particles. But all these experiments were performed when the different particles were mixed in about the same proportion. The question next came up: What will happen when the proportions are changed? Will Stentor select food particles from particles that are not food as accurately when the latter are greatly in excess? or in a mixture of different

kinds of food particles, will the ratio of ingesta vary directly with the proportion of the amounts of the different particles as they occur in the mixture? or does a variation in the different kinds of particles, in the matter of proportion, have no effect upon the amount of each kind ingested?

Trachelomonas volvocina, T. hispida, Phacus longicaudus, P. triqueter, Euglena viridis, and E. deses were placed in a small preparation dish with about 10 cc. of filtered Stentor culture solution. About 100 times as much carmine as food organisms was added and into this mixture several Stentors were placed, and the dish set in the dark for about an hour. Examination at the end of that time showed that about ten times as much food was eaten as carmine. This is practically the same result that was obtained in dish 3, described above, where the proportions of Euglena, Trachelomonas, and carmine were equal.

Another series of experiments bearing upon this point was performed with mixtures of a species of Euglena and a colorless flagellate, probably a species of Chilomonas. The Euglenæ were about 30μ long and about 12μ wide; Chilomonas, 18μ long and 8μ wide. These two organisms were mixed in various proportions. Stentors were then introduced and the dishes set in the dark for two hours. The contents of the dishes, together wi h the results obtained, follow:

1 Mixture of Euglenæ and Chilomonas in proportion of 4 : 1. Result: Ten times as many Euglenæ ingested as Chilomonas.

2 Chilomonas and Euglenæ in proportion of 4 : 1. Result: Four times as many Euglenæ ingested as Chilomonas. One typical Stentor contained 200 Euglenæ and 54 Chilomonas in 80 vacuoles.

3 Chilomonas and Euglenæ in same numbers. Result: About six times as many Euglenæ ingested as Chilomonas.

4 Many Chilomonas. Result: Stentors contained as many Chilomonas as Stentors in dish 6 contained Euglenæ.

5 Few Chilomonas. Result: Comparatively few Chilomonas eaten.

6 Many Euglenæ. Result: Many Euglenæ eaten,—from 5,000 to 11,000.

7 Few Euglenæ. Result: Comparatively few Euglenæ eaten.

Some peculiarities of behavior of the Stentors in the above series of experiments may be worth mentioning. About 60 per cent of the Stentors were swimming freely. In dishes 4 and 6 where the Chilomonas were dense, the Stentors swam continuously with the foot ahead. About one-third of all the free-swimming Stentors in dishes 2 and 4 (where the Chilomonas were dense) were in a state of almost maximum contraction. These Stentors contained very few Chilomonas. Nearly all the free-swimming Stentors in the other dishes were extended. Some of the attached Stentors in the dishes 2 and 4 were maximally contracted. These contained the smallest number of food organisms. Some of the other attached Stentors rolled masses of Chilomonas on the disk in the "push ball" fashion as described on page 86. The other attached Stentors in these dishes continually reversed their cilia. The attached Stentors in the Euglena dishes, 1, 3, 6 and 7, kept all the body cilia beating forward, whether all the Euglena were rejected or all eaten.

We have seen that Stentor takes less waste matter (carmine, etc.) when food is present, more when it is absent. This brings up another interesting question. In order that the animal shall reject substances not good for food, must it first have taken a certain amount of food? If so, how much food must be taken before discrimination begins? And in order to induce discrimination, is it necessary that food should be actually ingested, or is mere contact with food all that is required?

As to the quantity of food necessary to induce discrimination the experiments just described show that Stentor discriminates against carmine (or Chilomonas), about as completely when the proportion of Euglenæ present is very small (about 1 : 100) as when more Euglenæ are present. It is impracticable to work with smaller proportions of Euglenæ, since in such cases one cannot keep the various kinds of particles uniformly distributed.

To determine whether actual ingestion of food, or only contact with it, is necessary to induce discrimination, the following experiment was tried. The waste material, carmine, was mixed with rather large paramecia. The latter may serve as food for Stentor,

but they are rarely captured, though they frequently come in contact with the Stentors. Five dishes contained equal amounts of tap water and carmine particles; into some of these there was introduced also certain quantities of food particles, in others none. Fifty Stentors were placed in each of these dishes, and then were examined after 20 minutes to determine the relative amounts of carmine taken. The conditions and results are as follows.

1 Carmine alone. At the end of 20 minutes the Stentors contained carmine to the bulk of about 400 Euglenæ.

2 Several thousand paramecia plus carmine.

3 Several hundred paramecia plus carmine.

In dishes 2 and 3 none of the Stentors contained any paramecia. Their carmine content was about half that of the Stentors of dish 1, a bulk equivalent to that of about 200 Euglenæ.

4 Trachelomonas hispida, T. volvocina, and carmine. The Stentors contained a very few specimens of Trachelomonas. Carmine to the bulk of about 80 Euglenæ.

5 Euglena viridis and carmine. The Stentors had in 20 minutes taken each about 275 Euglenæ, and carmine equivalent in bulk to about 4 Euglenæ.

These results seem to indicate that the presence of the paramecia in dishes 2 and 3 decreased the amount of carmine taken up by the Stentors. The paramecia frequently touched the disk, and were often swept into the Stentors' pouches but always escaped, and it is probable that this touching of the discal or pouch cilia by the paramecia altered the physiologic condition of the Stentor in much the same way as if several paramecia had been eaten. It might be thought that the bacteria carried over with the paramecia produced the result described above, but this is probably not the case, since in many experiments of this nature carmine was put into a portion of filtered paramecia or Stentor culture solution which was full of bacteria, and yet the maximum quantity of carmine was taken up.

The evidence thus indicates that it is only necessary for food particles to touch the Stentor's disk or pouch in order that Stentor should then to some extent reject the waste particles.

A question related to the one we have just considered is the fol-

lowing. Does not Stentor react or behave differently to such indigestible particles as carmine or india ink after being in contact with them for a few minutes or half an hour? In other words, may not the Stentor under these conditions be "educated" into rejecting carmine after having for some time ingested this substance?

The paper by Metalnikow, referred to in the introduction, bears upon this very point. This author worked upon paramecium and found that if these organisms are fed with carmine for 15 days or so, they cease to take up this substance. "Füttert man Paramecien sehr lange Zeit hindurch nur mit einem Farbstoff, z. B. mit Karmin, so kann man häufig nachstehende interessante Erscheinung beobachten. Anfangs, während der ersten Tage des Fütterns, bilden sich in einem jeden Infusor 30-40 kleine, gaf ärbte, Karmin enthaltende Vacuolen. Hierauf nimmt die Zahl der Vacuolen immer mehr und mehr ab. Bereits nach wenigen Tagen enthält ein jedes Infusor nicht mehr wie 15-20 Vacuolen, während nach 10-15 Tagen nicht wenige Infusorien angetroffen werden, welche keine einzige gefärbte Vacuole mehr enthalten. Nach einem noch grösseren Zeitraum hören sämmtliche Infusorien auf Karmin zu verschlucken" (*l. z.*, p. 183, 184).

I attempted to verify Metalnikow's results upon Stentor and expected at the outset to find perhaps a greater capacity in Stentor to acquire such a "ganz neue Fähigkeit" than in Paramecium, because of the generally accepted notion that Stentor is more highly developed as far at least as its "action system" is concerned. It would be in line with the results obtained by my other experiments on Stentor. But I was disappointed, as the following experiments show.

In 5 large covered dishes were placed 60 watch glasses, each one being filled with about 5 cc. of fresh, weak, filtered hay infusion. In 6 of these glasses were placed Stentors; in 6 more were placed Stentors and paramecia; ratio, 1 :10; 6 others contained Stentors and Euglenæ, 1 :100; 6 others contained Stentors, paramecia, and india ink; 6 others, Stentors, paramecia, and carmine; 3 others Stentors and india ink; 3 others, Stentors and carmine; 6 others, paramecia; 3 others, paramecia and india ink; 3 others, paramecia and carmine; 6 others, paramecia and Euglena; 3 others, para-

mecia, Euglenæ, and carmine; 3 others, paramecia, Euglenæ, and india ink.

One hour after these experiments were set up all the glasses were examined. All the Stentors which were placed in ink or carmine mixtures, whether paramecia were present or not, contained ink or carmine but the amounts were variable. More carmine was found in the Stentors than ink. Likewise all the paramecia placed in ink or carmine mixtures contained vacuoles of these substances. The highest number of vacuoles was from 40 to 50. The lowest number observed was 4. More ink than carmine was found n paramecia.

All the glasses were examined once a day after they were set up. Three days after the experiment was begun, some paramecia were found with very few vacuoles of carmine. The smallest number was 4. These paramecia were then placed in fresh carmine, and in half an hour they contained 50 or more vacuoles each. The carmine and ink content of the Stentors had so far remained constant. Seven days after the experiment was set up, no decrease in the number of vacuoles of ink or carmine in either paramecia or Stentors was found. But one paramecium was found with no carmine vacuoles. This paramecium was then placed with fresh carmine and for over an hour no carmine was taken. It was then placed in fresh ink and in 30 minutes 6 vacuoles of ink were found to have been ingested. This result is probably due to the fact that the grains of india ink are smaller than the grains of carmine.

Ten days after the experiment was set up many paramecia in those glasses where the least ink and carmine happened to be, contained very few vacuoles of these substances, but upon adding some fresh carmine or ink these paramecia at once filled up on these substances as they had done in the beginning of the experiment. The paramecia in the glasses where the carmine or ink was dense contained about the same quantity of these substances as at the start. About the same may be said for the Stentors. In the glasses in which the ink or carmine was dense the Stentors were filled with these substances. In other glasses the Stentors contained very little ink or carmine; but upon refilling, these substances were again taken up as at the beginning of the experiment.

Fifteen days after the experiment was begun, Raphidium developed in the glasses and the Stentors died. About six of the dishes which contained paramecia and ink or carmine were however in good condition. These paramecia showed no decrease in the number of vacuoles of these substances. After two or three days these paramecia also died.

These results are quite at variance with those described by Metalnikow. I therefore contrived another experiment which eliminates certain possibilities of error to which the above experiment and those of Metalnikow were open. A clinostat was set up with a cylindrical jar revolving on the horizontal axis once in every 15 minutes. Seven small vials were then filled with Stentors and paramecia in 5 cc. of their own culture solution. In 3 of these vials was placed carmine; in the other 3 india ink; and the other was left for a control. The vials were corked and placed loosely in the jar revolved by the clinostat. As the clinostat revolved the jar, the vials rolled around, and as they did so their contents were shaken up thoroughly about three times every 15 minutes. In this way the contents of the vials were distributed uniformly for the thirty-three days throughout which the experiment was carried on. The Stentors, paramecia, and culture solution were placed in the vials in the same proportion in which they existed in nature. A little fresh carmine or ink was added every few days. The contents of the vials were examined every day, whenever possible, in this manner. The contents were poured out into a small preparation dish and placed under a binocular microscope. The condition of the Stentors and paramecia was then easily observable. In this way the water in the vials was also thoroughly aerated. About 30 Stentors and 500 paramecia were placed in each vial. The following are the tabulated results for the paramecia. The table shows the number of paramecia which contained no ink or carmine on the dates shown.

		OCTOBER								NOVEMBER												
DATES		22	23	24	26	27	28	29	30	31	4	5	6	7	9	10	11	12	13	16	18	20	23
Carmine vials	*a*	4	2	6	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	2	3	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	c	3	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	d	2	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0				
Ink vials	*e*	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0							
	f	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Many paramecia in the act of dividing were observed to have from 5 to 15 vacuoles in them. Only one paramecium was found to contain no ink or carmine in dividing.

The paramecia of November 4 and 12 which had no carmine or ink in them were of abnormal, boomerang shape. The number of vacuoles in the paramecia in these vials was almost constant as long as the experiment was carried on—over 33 days. Attempts were made at counting vacuoles and the average high number was about 70. About 5 per cent of the paramecia were found to conta n only from 5 to 15 vacuoles. In a number of cases such individuals were isolated and fresh carmine or ink added, when they would invariably fill up again. Ink was generally eaten more readily than carmine. The paramecia acted normally as long as the experiment was continued, and there was reason to believe that the experiment could have been continued further if it had been deemed necessary.

As for the Stentors, throughout the entire run of the experiment, none contained neither ink nor carmine. It was impossible to count the vacuoles because of their irregular sizes and shapes. The ink and carmine content of the Stentors was fairly constant. No decrease in the amounts of these substances in the Stentors was found throughout the entire experiment.

It is clear from these experiments that if either Stentor or paramecium can be "educated" in such a way as to refuse to eat carmine, etc., after feeding carmine to these organisms for a month, it can only be done under very special conditions. Metalnikow does not describe his experiments in detail, but from what

I was able to gather from his paper with regard to the method of work our results disagree utterly.

What is the reason for the disagreement? Metalnikow says that after his paramecia had ceased to take up carmine he stirred up the solution and yet the paramecia refused to ingest any more carmine. In my work I also found this to be true, but I attributed this to the fact that the carmine is no longer mixed in the same way as it was originally, for the mucus excreted by the paramecia, and other colloidal matter in the water, cause the carmine particles to stick together, so that the paramecia no longer take this kind of carmine with readiness. Further Metalnikow states that if one takes some of these paramecia which have refused to eat carmine and places them in fresh carmine, none is taken up. I have also tried this many times, but in almost every case I found that the paramecia again took up fresh carmine. In some few cases I found paramecia which did not eat carmine under any circumstances, even if they were placed in fresh solutions, but close examination nearly always showed that these rare individuals either were deformed or had just previously divided, and so were unable structurally to ingest the grains of fresh carmine. It appears possible that Metalnikow unfortunately got hold of some of these individuals.

But there is another statement in his paper which is hard to reconcile with the one quoted above.

"Hält man die Infusorien bei mässig hoher Temperatur (d. h. bei 10-15° C.; die Versuche wurden während der kalten Jahreszeit angestellt), so tritt während mehrere Tage keine Theilung ein.

"Jeden Tag untersuchte ich ein solches ungeteiltes Infusor auf seine Fähigkeit hin Karmin zu verschlucken. Im Verlauf von zwei, drei (in seltenen Fällen auch 4) Tagen, während welcher Zeit es mir gelungen war die Infusorien von einer Theilung abzuhalten, konnte ich nicht bemerken, dass ihr Verhalten zum Karmin irgend welche Abänderungen erfahren hätte. Jeden Tag weigerten sich die Infusorien hartnäckig Karmin zu fressen. Kaum aber hätte sich ein Infusor geteilt, so änderte sich sein Verhalten plötzlich und es begann von neuem Karmin zu fressen. In denjenigen Fällen wo ich das Infusor in den Thermostat oder an

einen warmen Ort (von über 20° C.) vebrachte, trat eine Theilung meistens sehr bald ein und die Tochterinfusorien begannen sofort Karmin körnchen zu verschlucken."

Metalnikow thus finds that although paramecia learn in some way to refuse carmine after being fed with it for some time, yet as soon as they divide, the daughter paramecia at once take up carmine as before. But he also says that after paramecia had been left in carmine for 15 days or longer, most of them contained no carmine whatever. Whether there were only very few paramecia dividing in this latter case, or whether the paramecia after several generations inherited this acquired character of refusing to ingest carmine, we are not told. These important details as well as others to which attention is called above, are not given in Metalnikow's paper, which is indeed only a preliminary paper. But until such details are made known, and until Metalnikow's experiments are described in detail, so that they may be verified, a discussion of our results which disagree in every essential particular, is futile.

THE BASIS OF SELECTION

A series of experiments will now be taken up which were designed for the purpose of ascertaining if possible upon what basis selection in Stentor is exercised. We have seen from the experiments up to this point that Euglenæ are eaten with more readiness than Phacus, and that carmine is more readily eaten than india ink, and that between living and dead organisms no selection seems to be exercised. What can be the basis of discrimination that gives such results? So far as we can tell there are only two possible methods by which Stentor can discriminate between two different substances; one is by a chemical sense ("tasting" and "smelling"), and the other by a tactual sense ("touching"). Does Stentor select a particle by "touching" it, or by "tasting it," or by both methods? (The psychological terminology is used here merely for convenience. By tasting and touching are meant reactions to chemicals and to contact and form, respectively.)

In the first experiments, organisms which are readily eaten by Stentor were altered as far as their chemical nature was concerned. Some were cooked, some soaked for a considerable time in alcohol, others in iodine, osmic acid, mercuric chloride, tannic acid, etc. Euglenæ, Trachelomonas, and very small paramecia were the organisms employed for these purposes. After they had been treated with the chemicals mentioned, they were thoroughly washed and sucked up into pipettes. Hungry Stentors were isolated; then normal living organisms, and also those treated with the chemicals, were fed to the Stentors in mixed order, as was done in the first series of experiments. In no case were the chemically treated organisms rejected while the living were eaten. Some Stentors ingested all the organisms, and others rejected some of the living and some of the chemically treated, while the rest, including organisms of both kinds, were rejected.

It was found in previous experiments that Stentor could discriminate between Phacus triqueter and P. longicaudus, and between Trachelomonas hispida and T. volvocina, etc. In the experiment just preceding Stentor did not discriminate between living organisms and organisms killed with osmic acid, iodine, etc. Now it seems improbable that Stentor selects upon a chemical basis, since it is hard to understand how there could be a greater difference in "taste" between living Phacus triqueter and living P. longicaudus, where there was selection, than between living Phacus triqueter and specimens of P. triqueter killed with acids, iodine, etc., where there was no selection.

But to test this result further another set of experiments was performed in which the form and surface texture of food organisms was changed to a greater or less extent, but the chemical nature was left, as nearly as possible, in the same state as in the normal organism. Paramecia and Euglenæ were used for these purposes. By means of very small platinum knives these organisms were cut into halves or quarters, or even smaller pieces, and then fed in a stream with whole living organisms of the same species. There was no discrimination. The pieces were eaten as readily as the whole specimens. Many of these organisms were then mashed and minced into very fine fragments so that the original form and

surface texture was quite destroyed. This "jelly" was then sucked up and fed to Stentors together with normal living organisms of the same species. The whole specimens were eaten but the "jelly" was rejected. The Stentors bent away or reversed their cilia the moment the mashed paramecia or Euglenæ touched the disk or pouch, exactly as they do when a cloud of carmine ink, etc., is similarly caused to come in contact with the Stentor.

Small starch grains were then mixed with this jelly and diluted and fed to the Stentors. No starch grains were eaten. The Stentors still reversed their cilia when any of this mixture touched the disk.

Starch grains (potato and corn) were then fed in solutions of various strengths of sugar, beef juice, pork juice, pepsin, Liebig's Extract of Beef. The starch was invariably rejected. This same thing was tried with carmine and india ink, but the same results were got here as were derived from the controls; no more carmine or ink was ingested if pork or beef juice, or sugar, was present, than when these were absent.

Still other experiments were carried on in which the food organisms were soaked in various chemicals such as iodine, anilin dyes, alcoholic solutions of quinine, etc., and then only part of the superfluous chemical was washed away, so that when fed, a little of the iodine, quinine, etc., would be in solution and thus give the Euglenæ or paramecia quite a different "taste" from that which these organisms normally possess. These experiments are very difficult to carry out, owing to the fact that when iodine or quinine is just a little too strong, the Stentors contract the moment these substances touch the disk, and yet if one washes these chemically treated organisms a little longer one cannot be sure that any iodine or quinine remains to affect the Stentor before the organisms are swallowed. The experiments are also somewhat uncertain, for in the case where an organism is eaten, we cannot be sure whether the quinine, dye, or iodine, etc., had any effect on the Stentor at all. But the following are the results. When the food organisms were not too vigorously washed, the Stentors that were fed with them bent away or contracted the moment the stream from the pipette touched the disk. When the washing was carried a little further the organisms were ingested as were the living organisms.

Now if Stentor selects its food upon a chemical basis we should expect something like the following to occur. 1. Living organisms ought to call forth a different reaction from those cooked or chemically treated, since it seems evident that the "taste" of these two classes of food is very different. 2. When living organisms are fed we should expect the behavior to differ from what occurs when they are fed in sugar solution, etc. 3. We should not expect carmine or ink to be eaten. 4. Starch grains would probably be eaten if soaked in, and fed with, paramecium juice; and the minute fragments of paramecia would also probably be eaten if Stentor selected its food upon a chemical basis. But as a matter of fact the reverse is true in each case.

But let us see what Stentor would probably do if it selected its food upon a tactual basis. Under tactual stimuli all those of size, weight, form, and surface texture, and discrimination might be made upon any one or all of these factors.

It is clear from the outset that size plays little or no part in the selection, for all sizes of organisms from bacteria to paramecia are ingested. So we have only to consider weight, form, and surface texture.

Selection upon the basis of weight alone would result in rejecting all substances which differed in weight (specific gravity) from living organisms. This would explain the rejection of glass, sand, starch, etc., and the ingestion of carmine and ink. But it probably would not explain selection obtaining between Phacus and Euglena or between Euglena and Chilomonas, or between a rapidly moving organism like Halteria and any other particle.

Selection on the basis of form alone would also fail to explain all the results obtained in the experiments. For a Phacus differs more from a Euglena in form than from a starch grain, etc.

Nor would the factor of surface texture completely explain all the results obtained in the experiments.

It seems clear therefore that as far as my experiments go, no single quality such as weight, or form, etc., is decisive for setting off the Stentor's ingesting mechanism in all cases where discrimination occurs. It is probable that more than one factor serves as a basis for discrimination.

But whatever the basis is, it is constantly varying. The degree of hunger, as we have seen, has a marked influence upon selection. Hunger perhaps does not form part of the real basis of selection inasmuch as it only influences the degree of accuracy in discrimination. The same statement may be made as regards mere contact with food. As was shown in experiments described on page 119, the mere contact of paramecia with Stentor seems, like hunger, to have increased the degree of accuracy in discrimination.

The experiments then in this part show that Stentor probably selects its food upon a tactual and not upon a chemical basis. Further the experiments seem to indicate that more than one of the factors, weight, form, or surface texture, serve as a basis for discrimination.

THE SELECTIVE MECHANISM

We have seen that there are two mechansims by means of which discrimination is directly brought about. The ingesting mechanism is set into action by certain qualities in particles, when the Stentor is hungry. The absence of these qualities, or the presence of qualities which are objectionable to Stentor, call into action the rejecting mechanism. These two mechanisms, taken each by itself, are constantly varying with regard to the strength of stimulus required to set either into action. As the Stentor passes from a hungry to a satiated stage the ingesting apparatus is continually less and less easily set off. But with the rejecting apparatus the reverse is true. As the Stentor grows more and more satiated, constantly weaker stimuli set off the rejecting mechanism until finally all particles stimulate it into action.

These changes are due to the constantly varying physiological state, brought about by the constantly accumulating food in the body of Stentor. That is, the amount of food ingested regulates solely the ease with which the ingesting or the rejecting mechanisms are set off. But in some cases there is evidently a direct effect produced in the stimulus-receiving organ by the stimulus. Such cases are those experiments in which carmine and food particles were fed, sometimes mixed, and sometimes each substance

by itself. Some Stentors from the beginning of the experiment rejected carmine apparently only because food was also present. The rejecting and the ingesting mechansism may therefore be acted upon and their mode of action in consequence changed, both by the physiological state as determined by the amount of food in the animal, and by the condition of the stimulus-receiving apparatus as influenced by the amount of previous stimulation.

Whenever a particle possesses qualities that stimulate to a greater or less extent both the ingesting and the rejecting mechanism, the action of the cilia in the pouch and funnel are not thoroughly coördinated either for ingesting or for rejecting, and as a consequence loops are formed in the path of the particle. It seems from the experiments that the stimuli from the objectionable features of a particle are summated more rapidly than the stimuli from the desirable features; for 80 per cent of all the particles that had loops in their paths were finally rejected. We also find that a much larger number of loops occur when the Stentor is beginning to be replete than when hungry or when almost satiated. At the stage when the animal is just beginning to be replete, stimuli of medium intensity are required to set off either the ingesting or the rejecting apparatus. The stimuli are more nearly of equal intensity, or in other words, the mechanisms are set off more nearly with equal readiness at this stage than at any other. This is probably the reason for the larger number of loops at this stage.

Selection between food and indigestible particles in Stentor is an almost perfect adaptation. With very few exceptions only particles (organisms) of food value are ingested. Indigestible particles of many sorts which have for thousands of generations not come in contact with Stentor are nevertheless rejected with accuracy.

SUMMARY

1 Stentor cæruleus exercises a selection among the particles that are brought to its food pouch by the ciliary current. The selection is brought about by changes in the beat of the cilia of the pouch and funnel. Certain particles are rejected by a localized

reversal of the cilia; others are carried to the mouth and ingested. Of two particles within the funnel at the same time one may be thus rejected while the other is ingested. Selection thus occurs not only from among particles reaching the pouch successively, but from a large number reaching the pouch at once.

2 Stentor discriminates very accurately between organisms (Phacus, Euglena, etc.) and indigestible particles (carmine, glass, sulphur, starch, etc.), ingesting the former and rejecting the latter.

3 Stentor discriminates between different kinds of organisms, eating some (Euglena, Phacus triqueter) with great readiness, while others (Trachelomonas hispida, Phacus longicaudus) are rarely ingested.

4 States of hunger and satiety, and intermediate conditions, are shown to exist in Stentor by differences in the behavior toward food. The animal discriminates more perfectly (i. e., more restrictively), when almost satiated than when very hungry. When very hungry it may ingest many indigestible particles (carmine, india ink, etc.)

5 The amount of a given substance ingested depends upon what other substances are present. Stentor in water containing indigestible particles, such as carmine, may ingest much of the latter; if the water contains in addition many organisms fit for food, very little of the indigestible matter is ingested.

6 It was not found that Stentor and paramecium become lastingly "educated" to reject certain sorts of food that they have formerly taken. Such changes as occur in selection seem to be mainly matters of hunger and satiety.

7 Stentor selects its food upon a tactual basis and apparently not upon a chemical one. That is, Stentor reacts in selecting food, to physical properties only or chiefly, and not to chemical properties.

8 Stentor in a condition of satiety differs in many respects from Stentor in a condition of hunger. In satiety we find the following conditions: *a.* Extension is always submaximal. *b.* The aboral side is more strongly convex than the oral side. *c.* There is a marked decrease in the activity of the membranellæ. *d.* Stentors respond much more readily to mechanical stimulation

at the disk. *e*. If the stimuli are submaximal, contraction is often only partly accomplished.

9 The amount of food in the body of the Stentor at any given moment is not an accurate register of the degree of satiety; the latter depends upon what recent stimuli have been received, as well as on the amount of food in the body.

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LIGHT AS A FACTOR IN THE REGENERATION OF HYDROIDS

SECOND STUDY

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Three different kinds of hydroids are found abundantly in the harbor of Woods Hole, Mass. Of these Tubularia (Parypha) crocea reaches its most luxuriant growth about the end of June and then declines in numbers and vitality toward the end of July or the first part of August, at which time they disappear completely. In the meantime Eudendrium ramosum begins to grow about the first part of July, reaches its maximum growth about the end of July and then gradually disappears. Pennaria tiarella comes last, toward the end of July, and persists until the beginning of September. All three kinds may be found on the same piles at the same time.

In a previous publication[1] the curious effect of light upon the regeneration of E. ramosum was described. From those studies it was evident that the idea of the simple and direct effect described by Loeb,[2] needed radical revision. In brief the facts for E. ramosum are as follows. Under ordinary conditions, hydranths are replaced in about 48 hours. As the hydranths live only a few days, they are replaced again and again by new ones. If the number produced on successive days be examined, it is found that the largest number are regenerated within a few days after their removal, and that the number steadily decreases on succeeding

[1] Goldfarb, A. J. Experimental Study of Light as a Factor in the Regeneration of Hydroids. Journ. Exp. Zoöl., vol. 3, 1905.

[2] Loeb, J. The Influence of Light on the Development of Organs in Animals. Pflüger's Archiv., Bd. 63, 1895.

days, until about the 13th day, after which no more hydranths are produced.

When these colonies were removed to a dark chamber, dark enough not to effect photographic plates, the number of hydranths produced in this chamber, and the rate of their development, was not materially altered. Regeneration took place in the dark as well, or almost as well, as in the light. But this statement holds true only during the first cycle[3]. For, after this period a profound change occurred, as a result of which these hydroids did not regenerate a single hydranth so long as they were kept in the dark. This treatment rendered them remarkably responsive to light, so that exposure always induced the regeneration of hydranths. But the surprising feature was the intense sensitiveness which hydroids so treated seemed to possess. Exposure to shaded light of 15 seconds sufficed to stimulate the regeneration of a whole series of hydranths.

No explanation of this peculiar relation to light was given. It cannot be said that E. ramosum is dependent upon light by virtue of substances produced by symbiotically associated organisms. Nor is it dependent directly upon light for its sustenance in the manner of plants.

The following experiments were undertaken with the idea of finding what influence light has upon the regeneration of the other two closely associated hydroids, to ascertain whether this extreme sensitiveness to light is common to the other forms, and possibly to determine what significance this fact has in the life history of these plant-like animals[4].

The procedure was practically the same in all the experiments. The hydranths were removed from the selected stalks and the same number were placed in wide shallow dishes containing the same quantity of sea-water. To prevent the rapid accumulation of bacteria, the water was changed at first daily, and in the later experiments, on alternate days. The controls were kept in the shaded light of the laboratory, the others were prepared, in the

[3] Vide Journ. Exp. Zool. Vol. 3, 1905.

[4] The experiments were made at the Marine Biological Laboratory, Woods Hole, Mass. For the privilege of working therein I am indebted to the Director, Prof. F. R. Lillie.

manner just described, and kept in a dark chamber. The manipulation, change of water and counting of hydranths of the latter stalks were all done in the dark room, into which the only light was that which came through a double thickness of ruby glass. The temperature of the controls varied slightly from the others, the difference never exceeding 2 to $2\frac{1}{2}^{\circ}$ C. As each new hydranth was fully developed it was removed. Thus the figures in the tables represent the actual number of regenerated hydranths.

TUBULARIA (PARYPHA) CROCEA

This is a colonial hydroid usually made up of unbranched stalks, though not infrequently small branches are developed. Stalks were cut off quite close to their bases, their hydranths were then removed at approximately the same level for all the stalks of a series, i. e., either at the base of the hydranth or the middle of the stalk, etc. The results obtained among the controls and among those maintained in the dark chamber are given in the accompanying table.

In Experiment 1, the hydranths were removed about five-eighths inch from the apical ends. No branching stalks were used. The 24 controls were prepared in the light, 40 others were prepared and kept in the dark. In Experiment 2, there were 20 controls, and 42 in the dark. In Experiments 3 and 4, all hydranths were removed in the light. In Experiment 3, there were 20 controls, 60 in the dark. In Experiment 4, the hydranths only were removed, leaving one part of a colony of about 50 stalks, connected at their bases, to serve as a control, and another part of the colony was placed in the dark. In all there were 114 controls and 192 in the dark.

As in E. ramosum, the hydranths are regenerated ordinarily within two days after their removal. In the dark, their first appearance was somewhat delayed, though all subsequent regeneration took place at the normal rate. This initial retardation is seen in Experiment 1, where 24 controls regenerated 21 hydranths on the second day and 1 on the third day. The 40 kept in the dark regenerated 5 on the second day, while 23 appeared on

TABLE I

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4	
	In the Dark	In the Light	In the Dark	In the Light	In the Dark	In the Light	In the Dark	In the Light
No. of stalks	40	24	42	20	60	20	50	50
	Regenerated		*Regenerated*		*Regenerated*		*Regenerated*	
2d day	5	21	4	14	1	3	0	0
3d	23	1	10	8	8	3	0	0
4th	6	5	3	2	4	7	0	0
5th	3	4	0	0	1	3	0	1
6th	3	0	3	0	3	1	1	2
7th	4	1	1	2	4	2	1	10
8th	1	0	1	1	3	0	6	5
9th	0	0	0	0	2	1	7	4
10th	2	0	0	0	0	1	7	2
11th	0	0	0	0	0	0	9	2
12th	0	0	0	1	2	0	0	4
13th	0	0	0	1	1	0	3	0
Total Reg.	47	32	22	29	29	21	34	30
Per cent Reg.	117	133	52	145	45	105	68	60
			Exposed 15 minutes					
14th	0	0	0	0	0	0	2	1
15th	0	1	0	0	0	0	4	0
	Exposed Permanently							
16th	0	0	4	0	0	0	3	0
17th	0	0	0	0	0	0	0	0
18th	2	0	0	0	1	0	2	0
19th	1	0	1	0	0	0	0	0
20th	0	0	0	0				
21st	1	0	0	0				
22d	0	0	0	0				
24th	0		0	0				
25th			0	0				

	TOTAL NUMBER OF STALKS	NUMBER REGENERATED	PER CENT REGENERATED
In the dark	192	132	68
In the light	114	112	99

the third day. In Experiment 2, 20 controls regenerated 14 and 8 hydranths on the second and third days respectively, while 42, in the dark, regenerated 4 on the second and 10 on the third day. The same relation obtains in Experiment 3. In Experiment 4, 50 controls regenerated 1, 2, 10, 5, 4, and 2 hydranths on the 5th, 6th, 7th, 8th, 9th and 10th days respectively, while the 50 in the dark regenerated 0, 1, 1, 6, 7, and 7 hydranths in the same time.

The curve representing the number of hydranths regenerated on successive days, are analogous and that given for Eudendrium. The maximum number produced at any one time appeared within a few days after the beginning of the experiment, and the number steadily decreased on the following days until no more were produced. In Experiment 3, no more hydranths appeared after the 10th day, in Experiment 2, after the 13th day, in Experiment 4, after the 14th day, and in Experiment 1, after the 15th day. Though the observations were continued for over 25 days the controls no longer produced any hydranths. In this respect Tubularia crocea behaves exactly like Eudendrium.

While the controls no longer regenerated after 7, 10, 13 and 14 days respectively, those kept in the dark produced new hydranths during 10, 13, 8 and 17 days for the corresponding series. This difference in time, namely, 3 days, was much greater than the initial retardation. This may be interpreted to mean that the prolonged darkness continued to retard the development of the hydranths.

Beside this retardation, the removal of light also inhibits development to a considerable degree. Even during the first cycle, while the number of hydranths that were regenerated was large, the maximum (maximum per cent rather than the absolute number is here referred to), for any one day was always less than among the corresponding controls. Also the total per cent regenerated during a definite period, 13 days for example, was about 31 per cent less in the dark, than in the light. Darkness then exerts certain definite and measureable effects even during the first period or cycle, and in these respects the behavior of this hydroid is not unlike that of Eudendrium.

But it is only after this first cycle, that the stalks kept in the dark

come to differ so radically from the controls. The latter by this time were quite spent, i. e., they no longer produced hydranths in the light. Those in the dark, likewise produced no more hydranths but upon exposure to light not a single but a series of hydranths were regenerated. In Experiment 1, and 2, 4 and 5 hydranths, respectively, were regenerated in this manner. An exposure of but 15 minutes sufficed to stimulate the production of several hydranths. In this respect also Tubularia closely resembles Eudendrium, though the latter was far more sensitive to light, as shown by the larger number of hydranths regenerated after exposures, by the longer regenerating period, and by the briefer light stimulus that sufficed to bring these about.

There are two minor points that may be mentioned at this place. (1) Individual records made it quite certain that stalks kept in the dark could regenerate a second and third time. (2) Colonies whose stalks had been separated behaved in exactly the same manner as those that were not so separated from the colony.

The experiments were drawn to a close by the lateness of the season, when good healthy stalks were no more to be procured. It would have been interesting to have ascertained with far more exactness the minimal exposure that would have stimulated a regenerative cycle, to have ascertained whether a second or third cycle could have been induced by such brief or briefer exposures.

But the facts, so far as they go, clearly indicate that Tubularia crocea behaves essentially like Eudendrium. During the first cycle, regeneration takes place in the dark almost as well as in the light; that after this period regeneration occurs only after the stalks are exposed to the light. The two hydroids differ in that longer exposures are required to stimulate Tubularia, and that fewer hydranths result from such stimulation; in other words Tubularia is less sensitive to light than Eudendrium. The uniformity of the results in the four experiments bespeaks the correctness of these conclusions.

PENNARIA TIARELLA

It has been already pointed out that this hydroid lives with T. crocea and E. ramosum on the same piles and at the same sea level. Like Eudendrium, it is very much branched, and experiments showed that the regeneration of similar pieces from these two hydroids gave practically the same results.

New hydranths were regenerated in about 48 hours after their removal, and in this regard resembled the other two hydroids. These newly formed hydranths were removed daily, so that the figures actually represent the number of different hydranths regenerated.

There is one disturbing factor that has to be reckoned with, namely, the tendency to produce "roots" in place of hydranths particularly after thigmotactic stimuli, such as contact with the side of the dish, or with other stems. In as much as the size of the stalks, of the dishes, the number of stalks in each dish and other conditions were quite the same among the controls and among those in the dark, this disturbing factor may be fairly assumed to be constant in both sets of stalks. In some colonies this tendency to root formation is so strong that nearly all cut ends produced roots instead of hydranths.

The regeneration of Pennaria differs from the other two hydroids in several respects. In the first place, the curve represented by the number of new hydranths on successive days, was not so definite as in Tubularia or Eudendrium, due in all probability to the tendency to heteromorphic "root" formation. Yet the curve of Pennaria approximated very closely that of the other two hydroids. This is shown as follows. The maximum number of hydranths produced on any one day, appeared during the early part of the experiment, and if continued for a long period the number towards the close of the experiment was always very small; and these hydroids, it might be added, were decidedly smaller, i. e., one-half to one-third the normal size. In Experiment 5, the number produced on the 2d to the 6th days inclusive, was 12, 15, 16, 13, 11 hydranths. During succeeding 5 day intervals, the greatest number that appeared on any one day was

13, 8, 4 and 2. Greater irregularities took place in Experiments 6 and 7. In Experiment 6, 8 was the largest number of hydranths present at one time during the first 5 days and during succeeding 5 day intervals the largest number was 14, 17, 9, 10, 5, 13, and 5 hydranths. In Experiment 7, the figures for the same intervals were 10, 10, and 7 hydranths.

In the second place, new hydranths were regenerated during a longer cycle than either of the other two hydroids, 23 days in Experiment 7, 26 days in Experiment 5, and 35 days in Experiment 6, and would in all probability have continued to regenerate for a longer period. After so protracted an interval the hydranths were not only fewer as mentioned above but were decidedly smaller or malformed.

The most decided difference was observed in the behavior of those stalks that were placed in the dark. From the first not a single hydranth was regenerated from these stalks. Although a little over one thousand branchlets and pedicels from various colonies were placed in the dark, in no instance was a hydranth produced. Pennaria unlike Tubularia and Eudendrium requires no preliminary treatment in order to bring about a total cessation of the regenerative processes.

As might have been anticipated, the stalks in the dark required particularly long exposures in order to stimulate the formation of new hydranths. Exposures of 2 minutes and 5 minutes (Experiment 5) proved totally inefficient. Exposures of 3, 5, 8, 10, 15, 20, 25, 30, and even 60 minutes (Experiment 6) were equally inadequate, even though the exposures were made in the direct rays of the sun. Exposures of 2 hours, 3 hours and 4 hours were sometimes ineffective and sometimes produced hydranths. In Experiment 6, for example, 200 pedicels exposed for 2 hours regenerated during the next five days only 4 hydranths, while the controls regenerated 70 hydranths in the same time. Exposures of 3 and 4 hours gave no better results than the 2 hour exposures. The hydranths so produced were frequently dwarfed. The minimal time required to stimulate regeneration could be still further reduced, in some colonies, by the simple expedient of exposing the hydroids daily. Instead of two or more hour exposures, regenera-

tion could be induced with far more certainty by exposures of one hour or one-half hour for several successive days. And although very few hydranths were produced at any one time they appeared on as many as 5 separate days. Thus, daily half hour exposures resulted in 0, 0, 3, 6, 0, hydranths in one set of stalks and 0, 0, 1, 1, 1, 0 in another set. One hour exposures daily gave rise to 0, 0, 0, 1, 1, 0 and 0, 0, 0, 4, 0, 0, and 0, 0, 2, 1, 2, 2 hydranths in 3 different sets of stalks.

If left in the light for a whole day, hydranths were almost certain to appear in every experiment and these were large and numerous. Though the absence of light was inimical to development yet no permanent injury resulted from prolonged retention in the dark. For on returning the stalks to the light many normal sized hydranths were immediately regenerated. After 10 days in the dark the stalks in Experiment 5, were brought into the light and produced 0, 1, 4, 8, 0, 5, 5, 1, 0, 3, 0, 2, 0, 4, 1, 3 hydranths during the next 16 days. In Experiment 6, after 16 days in the dark 0, 14, 31, 26, 68, 17, etc., hydranths were regenerated on successive days,

There is a variety of Pennaria that is found attached to eel grass not far from shore. This is said to belong to the same species as that found on the much more shaded piles of the wharves, though it differs from it in several respects. Some experiments were made upon this variety, to determine whether the difference in habitat was reflected in a difference in the amount of light required to stimulate regeneration. The stalks of this variety, it was found, required as a rule longer periods of exposure to stimulate the development of new hydranths. In other respects their behavior was quite the same.

Loeb[2] in his account of the regeneration of Eudendrium ramosum stated that this hydroid never regenerated in the absence of light. He undoubtedly confused Eudendrium with Pennaria tiarella. In conversation with the writer Loeb expressed the opinion that this was probably the case.

These comparative studies make it perfectly clear that so remarkable a sensitivenes as that displayed by Eudendrium (after the first cycle) finds no parallel among the other two closely

associated hydroids. On the other hand the absence of light is not so directly preventive of hydranth formation among Tubularia and Eudendrium as in Pennaria. These hydroids living practically in the same environment agree in that after they have ceased to produce hydranths they may be stimulated to regenerate them by light and vice versa its absence retards and ultimately inhibits development. But the conditions and the degree to which light is effective varies with each hydroid. In Parypha and Eudendrium the absence of light inhibits regeneration only after a prolonged preliminary period. In Pennaria it is a coditio sine qua non from the very beginning.

SUMMARY

Light is a well defined factor in the regeneration of these hydroids, but the degree of effectiveness and the duration of the preliminary period required to render the hydroids susceptible to light stimuli varies. Eudendrium ramosum has a long preliminary cycle during which regeneration takes place in the dark almost as well as in the light. After this period, no regeneration occurs so long as stalks are maintained in the dark. A very brief stimulus, that is, an exposure to the light of 15 seconds, sufficed to call forth a series or cycle of hydranths. New series of hydranths could be produced again and again by repeated exposures. Like Eudendrium, Tubularia crocea also has a preliminary period of about 13 days during which hydranths are developed almost as well as in the light. At the expiration of this cycle, regeneration may be stimulated by exposure to the light of about 15 or more minutes. Pennaria tiarella differs from the other two hydroids in that there is no preliminary cycle. From the beginning hydranths are never regenerated in the dark. They may be stimulated to develop only by long exposures of 2 hours or more, or by exposures of one-half to one hour daily.

FURTHER STUDIES OF THE PROCESS OF HEREDITY IN FUNDULUS HYBRIDS[1]

H. H. NEWMAN

With Seven Text-Figures

I. THE INFLUENCE OF THE SPERMATOZOÖN ON THE RATE AND CHARACTER OF EARLY CLEAVAGE

In a previous paper on Fundulus hybrids[2] it was stated that the developmental rhythm of the young embryo was distinctly influenced by the foreign spermatozoön as early as fourteen hours after fertilization. It was also conjectured that the influence of the male cell was operative at a much earlier period, although it did not manifest itself in a measurable degree. Attempts were made to test the influence of the foreign sperm upon the early cleavage rhythm, but the results were largely negative owing to the crudeness of the methods employed.

As the writer was convinced that these results needed reëxamination, the work was resumed during the summer of 1909. This time the methods proved to be sufficiently refined to suit the case and positive results were obtained. The treatment was statistical in the sense that very large numbers of eggs were examined, and this involved an amount of tedious labor and eye-strain probably out of proportion to the value of the results obtained.

During the earlier attempts much difficulty was experienced in obtaining satisfactory data concerning the rate of cleavage. It seems a simple enough matter, to one who has not made the attempt, to enumerate the 2- and 4-cell stages in a batch of a thousand eggs or more. In reality, however, the difficulties are numer-

[1] Contributions from the Zoölogical Laboratories, University of Texas, No. 100.

[2] Journal of Experimental Zoölogy, vol. v, no. 4.

ous and not entirely surmountable for reasons that will soon become clear.

A large percentage of the eggs show intermediate stages between the 2- and the 4-cell condition, and in many cases the first two blastomeres undergo the second cleavage at different rates, producing 3-cell conditions. In earlier studies all eggs were counted as 4-cell stages if the second cleavage had begun, thus grouping into one class all stages from the end of the 2-cell to the beginning of the 8-cell condition. In practically all of the earlier experiments the additional error was made of allowing the development to proceed a little too far, so that nearly all of the eggs had at least begun the second cleavage. In later stages (8-, 16-, and 32-cell conditions) it became a matter of great difficulty to assign the various individuals to one of two classes, for the reason that cleavage is far from regular either in time rate or the arrangement of the cells.

In all experiments described in the previous paper, where the eggs of Fundulus majalis were involved, the results show a slightly more rapid rate of cleavage in the hybrid than in the pure-bred eggs. The eggs of F. majalis were found to be much more suitable for a statistical treatment of the rate of cleavage than those of Fundulus heteroclitus because of their larger size, the greater contrast between the color of the yolk and of the blastomeres, and also, a matter of extreme importance, the fact that the eggs if fertilized in a small amount of water, remain entirely separate from one another and can be kept evenly distributed in a single layer on the bottom of large flat dishes. All eggs can thus be kept under identical conditions, so far as illumination, temperature, and oxygen supply are concerned. The eggs, moreover, are not sticky and can readily be handled with needles or pipette. Those of F. heteroclitus, on the other hand, invariably cohere in clumps so that the innermost eggs are more or less cut off from light and oxygen. It is possible, of course, to separate these clumps, but the operation is a slow and tedious one and before it can be completed many eggs will have been hindered in their development. Even after these eggs have been separated they are difficult to handle as they remain somewhat sticky for a long

time and tend to adhere in a very unpleasant way to the instruments used for handling them. For these reasons, the eggs of F. heteroclitus are deemed unfit for experiments of the present sort and attention has been given solely to the eggs of F. majalis.

The species F. majalis develops only about two-thirds as rapidly as the species F. heretoclitus, the former reaching the hatching period in about three weeks; the latter in about two weeks. The same general time ratio prevails throughout the entire developmental process. In F. majalis the second cleavage occurs in about four hours after fertilization, while in F. heteroclitus it occurs in about three hours. Hybrid eggs (F. majalis ♀ F. heteroclitus ♂) show a slight acceleration of early cleavage as compared with pure-bred F. majalis eggs.

Expecting the influence of the foreign spermatozoön to be very slight at so early a period as the second cleavage, a very large number of eggs were used and as many experiments were performed as the time would permit.

In all experiments it was found necessary to distinguish several stages between the 2- and the 4-cell conditions. As a rule the following stages could be distinguished:

a. Complete 4-cell, shamrock-shaped, with each blastomere separated from its neighbors by a deep notch (Fig. 7).

b. Incomplete 4-cell, with the notches of the second cleavage less deep than those of the first (Fig. 6).

c. 3-cell, in which the second cleavage had occurred in one of the blastomeres and not in the other.

d. 2-cell with second cleavage furrow just beginning (Fig. 5).

e. Complete 2-cell, with the blastomeres separated by deep notches (Fig. 4).

f. Incomplete 2-cell, with the first cleavage just beginning or incomplete (Figs. 1, 2 and 3).

For the sake of brevity these stages are designated as follows: 4-cell, 4-minus, 3-cell, 2-plus, 2-cell, 2-minus; the 4-minus being valued at $3\frac{1}{2}$, 2-plus at $2\frac{1}{2}$, the 2-minus at $1\frac{1}{2}$, and the rest at face value.

Eggs that show no signs of cleavage are designated as "uncleaved." The majority of the latter have either failed by chance

to be fertilized or are incapable of development. A few might develop if allowed more time. No attempt, however, has been made to distinguish between unfertilized and retarded eggs, as cytological methods would be required.

In all experiments every precaution was taken to treat the lots of eggs used for pure and hybrid strains exactly alike. In some of the experiments a mixed lot of eggs from several females and the mixed milt of several males of each species yielded good results, while in other cases the eggs of one selected female were divided and fertilized by the milt of two or more selected males of each species. In every instance the eggs after stripping were thoroughly mixed by stirring and shaking and then divided into two approximately equal lots, which were fertilized at the same instant by abundant milt obtained by macerating the ripe testes of selected males. When development had proceeded to the desired point the two lots, pure and hybrid, were killed at the same instant in equal amounts of picro-sulphuric acid. It was found advantageous to examine and count the eggs while in this killing solution because the clear definition of the blastomeres is lost if the eggs are transferred to alcohol. To obtain the best results the examination and enumeration of eggs should be made within a few days after killing.

After the counts have been made there are obviously several methods of dealing with the data thus obtained. The best method involves a more or less arbitrary valuation of the various stages in terms of blastomeres, the uncleaved and unripe eggs being omitted from consideration. The total number of blastomeres divided by the number of eggs will give the average condition of the lot and will furnish a numerical comparison between the stages of advancement of the pure and hybrid strains. A simpler method consists of a mere comparison of the relative percentage, in the two lots, of more and less advanced conditions, the most significant comparison being that between all 4-cell stages (including 4-minus) and all 2-cell stages (including 2-plus and 2-minus), 3-cell condition being ignored because not strictly assignable to either class. Another simple method involves a comparison of 4-cell, 2-cell and intermediate stages in the two strains. A full

account of a fourth and considerably more searching method of comparison, which was used in the last two experiments, precedes Experiment 6.

The three methods of comparison just described were used in the first four experiments and were in each case mutually confirmatory. For the sake of brevity and uniformity they will, in each experiment, be designated as follows:

Method I. Comparison of average number of blastomeres.

Method II. Comparison of all 4's and all 2's.

Method III. Comparison of strict 4's, strict 2's and intermediates.

EXPERIMENTAL DATA

Experiment 1

Eggs of several females and milt of two males of each species used. Killed three hours and forty-five minutes after fertilization.

TABLE I

STAGE	VALUE	PURE-BRED		HYBRID	
		No. of Eggs	No. of Blastomeres	No. of Eggs	No. of Blastomeres
4-cell	4	74	296	167	668
4-minus	3½	62	217	65	227½
3-cell	3	33	99	23	69
2-plus	2½	56	140	28	70
2-cell	2	101	202	64	128
2-minus	1½	3	4½	6	9
Total		329	958½	353	1171½

There were 235 uncleaved eggs in the pure-bred lot and 218 in the hybrid.

Method I. Comparison of average number of blastomeres. The average in the pure-bred lot is 2.91 +; that in the hybrid lot 3.31 +.

Method II. Comparison of all 4's and all 2's. In the pure-bred lot there are 41.33 + per cent of 4's and 48.63 + per cent of 2's; in the hybrid lot 65.72 + per cent of 4's and 27.76 + per cent of 2's.

Method III. Comparison of strict 4's, strict 2's and intermediates.

In the pure-bred lot there are 22.49 + per cent strict 4's, 30.69 + per cent strict 2's and 45.89 + per cent intermediates; in the hybrid lot 47.30 + per cent of strict 4's, 18.13 + per cent of strict 2's and 32.86 + per cent of intermediates.

The per cent of 2-minus conditions is too small in both strains to furnish a basis of comparison.

Experiment 2

Eggs of several females and milt of several males of each species used. Killed three hours and fifty-five minutes after fertilization.

TABLE II

STAGE	VALUE	PURE-BRED		HYBRID	
		No. of Eggs	No. of Blastomeres	No. of Eggs	No. of Blastomeres
4-cell	4	86	344	336	1344
4-minus	3½	85	297½	125	437½
3-cell	3	19	57	30	90
2-plus	2½	40	100	71	106½
2-cell	2	44	88	76	152
2-minus	1½	18	27	23	35½
Total		292	913½	661	2165½

Method I. Comparison of average number of blastomeres, the average in the pure bred-lot is 3.13 +; that in the hybrid lot 3.27 +.

Method II. Comparison of all 4's and all 2's. In the pure-bred lot there are 58.56 + per cent of 4's and 34.93 + per cent of 2's; in the hybrid lot 69.74 + per cent of 4's and 25.71 + per cent of 2's.

Method III. Comparison of strict 4's, strict 2's and intermediates.

In the pure-bred lot there are 29.45 + per cent of strict 4's, 15.06 + per cent of strict 2's and 49.31 + per cent of intermediates;

in the hybrid lot, 50.83 + per cent of strict 4's, 11.49 + per cent of strict 2's and 34.19 + per cent of intermediates.

The percentage of 3-cell and of 2-minus conditions is somewhat smaller in the hybrid than in the pure-bred strain.

There were 442 uncleaved eggs in the pure-bred lot and 222 in the hybrid lot. It often happens that there is a larger percentage of heterogenic than of homogenic fertilizations. At present the factors governing this condition are not sufficiently understood to warrant a discussion.

Experiment 3

Eggs of a considerable number of females and milt of two choice males of each species used. Killed four hours and forty minutes after fertilization.

TABLE III

STAGE	VALUE	PURE-BRED		HYBRID	
		No. of Eggs	No. of Blastomeres	No. of Eggs	No. of Blastomeres
4-cell	4	275	1100	441	1764
4-minus	3½	560	1960	565	1977½
3-cell	3	74	222	76	228
2-plus	2½	138	345	76	190
2-cell	2	236	472	117	234
2-minus	1½	0	0	2	3
Total		1283	4099	1277	4396½

There were 397 uncleaved eggs in the pure-bred lot and 146 in the hybrid lot.

Method I. Comparison of the average number of blastomeres. The average in the pure-bred lot is 3.19 +; that in the hybrid lot 3.44 +.

Method II. Comparison of all 4's and all 2's. In the pure-bred lot there are 65.08 + per cent of 4's and 29.15 + per cent of 2's; in the hybrid lot 78.77 + per cent of 4's and 15.11 + per cent of 2's. The percentage of 3-cell stages is about equal in the two strains.

Method III. Comparison of strict 4's, strict 2's and intermediates.

In the pure-bred lot there are 21.42 + per cent of strict 4's, 18.40 + per cent of strict 2's and 60.17 + per cent of intermediates; in the hybrid lot 34.53 + per cent of strict 4's, 9.16 + per cent of strict 2's and 56.14 + per cent of intermediates.

Development has been allowed to proceed too far to show many stages below the 2-cell condition. The hybrid strain shows two eggs in the 2-minus condition and thus exhibits a wider range of variability even at so early a period as this.

Experiment 4

Eggs of several females divided and fertilized with the milt of three selected males of each species. Killed three hours and fifty minutes after fertilization.

TABLE IV

STAGE	VALUE	PURE-BRED		HYBRID	
		No. of Eggs	No. of Blastomeres	No. of Eggs	No. of Blastomeres
4-cell	4	32	128	41	164
3-plus	3½	88	308	109	381½
3-cell	3	52	156	60	180
2-plus	2½	75	187½	60	150
2-cell	2	312	624	284	568
2-minus	1½	28	42	12	18
Total		587	1445½	566	1461½

On account of comparatively low temperature cleavage proceeded somewhat more slowly in this than in preceding experiments; hence the much smaller proportion of the more advanced stages.

There were 253 uncleaved eggs in the pure-bred lot and 236 in the hybrid lot.

Method I. Comparison of the average number of blastomeres. The average in the pure-bred lot is 2.46 +; that in the hybrid lot 2.58 +.

Method II. Comparison of all 4's and all 2's. In the pure-bred lot 20.44+ per cent of 4's and 70.69 + per cent of 2's; in the hybrid lot 26.50 + per cent of 4's and 62.89 + per cent of 2's. There is only a slight difference in the percentage of 3-cell stages in the two strains.

Method III. Comparison of strict 4's, strict 2's and intermediates. In the pure-bred lot there are 5.45 + per cent of strict 4's 53.15 + per cent of strict 2's and 36.62 + per cent of intermediates; in the hybrid lot, 7.24 + per cent of strict 4's, 50.17 + per cent of strict 2's and 40.45 + per cent of intermediates. The percentage of 2-minus stages is more than twice as large in the pure-bred as in the hybrid strain.

Experiment 5

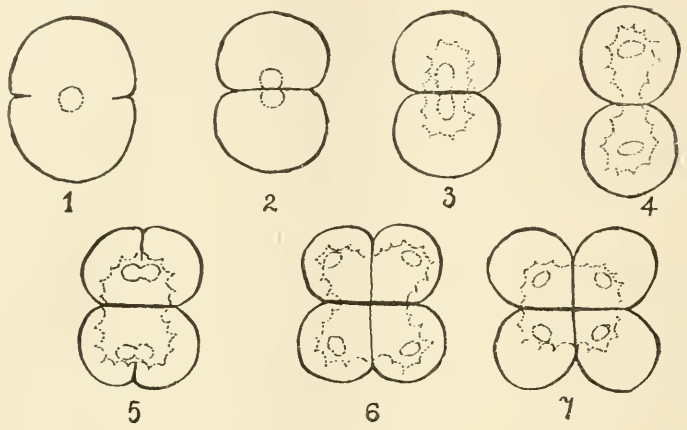
The eggs of several females divided and fertilized with the milt of two males of each species. Killed four hours after fertilization.

In this case a surprisingly small number of the pure-bred eggs developed, while a large number of the hybrid eggs cleaved normally. On account of the very small numbers of developing pure-bred eggs only the first method of comparison will be used. The average number of blastomeres in the pure-bred lot is 2.67 +; that of the hybrid lot 3.61 +; showing a marked difference in the same direction as in more successful experiments.

Experiment 6

In order to make the comparison between the pure-bred and the hybrid strains more searching, the eggs of one large female were divided into two approximately equal lots and fertilized with the mixed, expressed milt of several males of each species. Careful drawings had previously been made of seven stages between the 1-cell and the 4-cell conditions. These were made at approximately equal time intervals and were numbered from 1 to 7, beginning with the earliest stage. All eggs, so far as was possible, were examined and grouped according to their resemblance to the figures. In case an egg falls between two figured stages it is assigned to an intermediate group, e. g., an egg that falls between

stages 4 and 5 is classed as $4\frac{1}{2}$. The average condition can then be readily obtained by assigning to each egg a value corresponding to the figured group to which it belongs and dividing the total of these values by the total number of eggs. The result should give a very accurate numerical statement of the relative rate of cleavage in the two strains. The accompanying figures and table give in abbreviated form the results of the experiment.



Figs. 1-7

The average stage of the pure-bred lot is 5.11 +; that of the hybrid lot, 5.57 +, a difference of nearly half a stage.

Another method of dealing with these data is to compare the percentage of eggs assigned to the lower half and the upper half of the table, allowing the dividing line to fall below stage 5.

This shows a predominance of the more advanced stages to be more marked in the hybrid than in the pure-bred strain.

The mode in both cases is stage 6, but there is greater skewness of curve toward the 7 end of the curve in the hybrid than in the pure-bred array.

Another point worthy of note is that there is a much larger percentage of irregular cleavages in the hybrid lot than in the pure-bred. This in itself might be used as evidence of the very early formative influence of the spermatozoön.

The proportion of 3-cell stages is very nearly equal in the two

strains and has not been considered in the calculations because this condition is attained by only a comparatively few eggs and these could not be assigned to any of the figured classes.

TABLE V

FIGURED STAGES	PURE-BRED		HYBRID	
	No. of Eggs	Value in Terms of Stages	No. of Eggs	Value in Terms of Stages
1	2	2	3	3
2	2	4	2	4
3	3	9	1	3
3½	2	7	0	0
4	3	12	1	4
4½	12	54	5	22½
5	13	65	11	55
5½	17	93½	14	77
6	27	162	38	228
6½	1	6½	10	65
7	2	14	9	63
Total.....	84	429	94	524½

TABLE VI

	PURE-BRED		HYBRID	
	No. of Eggs	Per cent of Eggs	No. of Eggs	Per cent of Eggs
Below 5.....	25	28.57 +	12	12.76 +
5 and above.....	60	71.42 +	82	87.23 +

Experiment 7

As it became difficult to obtain any more good material it seemed advisable to reëxamine the eggs of one or two earlier experiments, using the more refined methods described in the last experiment.

One hundred eggs of each strain were drawn out at random by a disinterested person from the material used in Experiments 2 and 4. Each egg was assigned to its class and the average condition determined as before.

The material from Experiment 2 showed the average condition of the pure-bred eggs to be 5.26 +; that of the hybrid, 5.39 +.

The material from Experiment 4 showed the average condition of the pure-bred eggs to be 4.72 + that of the hybrid 4.76 +. The difference here is very slight, but in the same direction as in the other cases. No doubt another random selection of eggs from the material used in Experiment 4 would have shown a more marked difference than that just recorded.

Summary of Experimental Data and Conclusions

The tabular summary, Table VII, of the first four experiments will enable the reader to see at a glance that, no matter what method of comparison is used, there is a developmental balance in favor of the hybrid strain.

The last two experiments, somewhat more searching in character, show that the hybrid strains develop more rapidly than the pure bred.

In all cases the accelleration in developmental rhythm must be due the introduction of the spermatozoön of the more rapidly developing species.

In Experiment 6 it is clear, in addition, that the *form* of cleavage was affected by the foreign spermatozoön, in that there was a strong tendency toward irregularity in cleavage even in the early stages described.

We conclude that the male germ cell begins to exercise its hereditary function at a far earlier period than is commonly supposed.

II THE RÔLE OF THE SPERMATOZOÖN IN EARLY DEVELOPMENT

The question of the precise rôle of the male cell in early development has received much attention of late. It has come to be recognized that the spermatozoön has two separate functions, that of initiating development and that of imparting to the offspring the characters of the male parent. There seems to be a strong tendency today to regard the hereditary function as one that operates only after a period of abeyance, during which the hereditary characters of the young embryo are determined solely by the structure

TABLE VII

METHOD OF COMPARISON	SUBJECT OF COMPARISON	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4	
		Pure	Hybrid	Pure	Hybrid	Pure	Hybrid	Pure	Hybrid
I	Number of developing eggs	329	353	292	661	1283	1277	587	566
	Average number of blastomeres	2.91+	3.31+	3.13+	3.27+	3.19+	3.44+	2.46+	2.58+
	Excess in average number of blastomeres in favor of hybrid strain		.40		.14		.25		.12
II	Per cent of all 4-cell stages	41.33+	65.72+	58.56+	69.74+	65.08+	78.77+	20.44+	26.50+
	Per cent of all 2-cell stages	48.63+	27.76+	34.93+	25.71+	29.15+	15.11+	70.69+	62.89+
	Excess in per cent of all 4-cell stages in favor of hybrid strain		24.39		11.18		13.69		6.06
III	Per cent of strict 4-cell stages	22.49+	47.30+	29.45+	50.83+	21.42+	34.53+	5.45+	7.24+
	Per cent of strict 2-cell stages	30.69+	18.13+	15.06+	11.49+	18.40+	9.16+	53.15+	50.17+
	Per cent of intermediate stages	45.89+	32.86+	49.31+	34.19+	60.17+	56.14+	36.62+	40.45+
	Excess in per cent of strict 4-cell stages in favor of hybrid strain		24.81		21.38		13.11		1.79

of the egg protoplasm. This point of view has been clearly expressed by one of its leading exponents[3] in the following words:

"Finally as evidence that inheritance may take place through the cytoplasm of the egg, reference must be made to the extremely important work of Loeb and Godlewski. By concentration of hydroxyl-ions Loeb found that it was possible to cause the spermatozoa of starfishes and ophiurans to fertilize the eggs of sea-urchins. The embryos and larvæ resulting from such crosses showed only the characteristics of the mother. Later Godlewski, using the same methods, was able to fertilize the eggs of a sea-urchin with the sperm of a crinoid, and although such hybrids were raised to the larval stage, they showed only maternal characteristics. Still more, enucleated urchins eggs fertilized by crinoid sperm produced gastrulæ of purely urchin type. These results demonstrate, as Boveri admits, that the chromosomes of the sperm do not in this case influence or modify the cytoplasm of the egg cell; while the experiments on the enucleated egg show that the characteristics of the organism, at least as late as the gastrula stage, are derived entirely from the egg cytoplasm.

"Boveri long since showed that the early stages of development, perhaps as late as the blastula or gastrula, are uninfluenced by the spermatozoön and are purely maternal in type; in the case of Godlewski's hybrid larvæ, he supposes that the sperm chromosomes remain permanently inactive. But however this result is to be explained, it may be considered as definitely settled that the early development of animals is of purely maternal type, and that it is only in stages later than the gastrula, and consequently after the broad outlines of development and the general type of differentiation have been established, that the influence of the spermatozoön begins to make itself felt; and it is equally certain that this type of differentiation is predetermined in the cytoplasm of the mature egg cell, rather than in the egg nucleus.

"On the other hand, there is no doubt that the differentiations of the egg cytoplasm have arisen, in the main, during the ovarian history of the egg, and as a result of the interaction of

[3] Conklin, E. G. Science, N. S., vol. 27, no. 168, p. 98.

nucleus and cytoplasm; but the fact remains that *at the time of fertilization the hereditary potencies of the two germ cells are not equal, all the early development, including the polarity, symmetry, type of cleavage, and the relative positions and proportions of the future organs being predetermined in the cytoplasm of egg cell, while only the differentiations of later development are influenced by the sperm. In short, the egg cytoplasm fixes the type of development and the sperm and egg nuclei supply only the details.*"

My own observations on the early stages of the process of heredity and an examination of the experimental evidence, that lies at the foundation of the above view, together with a number of more recent contributions along the same line, force me to take a position on certain questions decidedly opposed to that of Conklin.

Is the specific symmetry, polarity, etc., expressed solely in the egg and not in the spermatozoön or in the various types of somatic cells? It is scarcely necessary to point out that the sperm cell at all stages of development shows just as pronounced a polarity as the egg—more so in later stages. This polarity is largely the expression of a definite relationship between nucleus and cytoplasm and is doubtless specific and hence characteristic of all cells of a given organism. No doubt this polarity expresses itself in a somewhat different fashion in different kinds of cells, but these special manifestations are, I believe, of secondary importance. When the spermatozoön for example, undergoes an exaggeration of its specific polarity and symmetry during the end stages of its development, when most of its cytoplasm is converted into a locomotor mechanism, it becomes a specialized cell with a definite function, and departs from the specific cell type. Is not the same true of the egg, a cell in which the primitive specific polarity and symmetry have been distorted by the large accumulations of inert nutritive material? It is entirely probable therefore, as Lillie has shown, that the real polarity and symmetry are characters of the ground substance common to all of the cells of the organism.

Since then a fertilized egg is a product of the more or less complete fusion of two cells with the same inherent specific polarity and symmetry, it appears somewhat extreme to state that "all

of the early development, including the polarity, symmetry, type of cleavage, and the relative positions and proportions of the future organs are predetermined in the cytoplasm of the egg."

Is it "definitely settled that the early development of animals is of purely maternal type, and that it is only in stages later than the gastrula, and consequently after the broad outlines of development and the general type of differentiation have been established, that the influence of the spermatozoön begins to make itself felt"?

The experiments detailed in an earlier part of this paper show that the spermatozoön exercises an hereditary influence upon the rate of development at the earliest possible period when it could be noticed or measured, and would seem to indicate that the hereditary function of the male germ cell begins to operate immediately, not after a period of abeyance.

Godlewski has also shown in his hybrids that there is a well marked retardation in the cleavage as early as the 4-cell stage.

There is evidence also that the form of cleavage is subject to the influence of the spermatozoön, as was indicated in Experiment 6, where in the hybrid strain there was a preponderance of irregular cleavages. This phenomenon is seen to much greater advantage in another cross, produced by fertilizing the eggs of Cyprinodon variegatus with the sperm of Fundulus heteroclitus. In this case the whole cleavage is decidedly irregular after the 4-cell stage.

Fischel[4] has shown that in a number of Echinoderm hybrids the male influence is expressed structurally in the early blastula stages, not only in the general size of the embryos, but in the actual size and shape of the cells. The sperm also seems to be responsible for the production of a number of early monstrosities in which the "broad outlines of development" have been decidedly distorted. Such typical monstrosities are very common among Fundulus hybrids and there is no doubt that similar conditions are found in all hybrid experiments.

Another phenomenon that has caught the attention of many observers is the wide range of variability among hybrids. This

[4] Archiv. f. Entw. Mech., vol. 22, pp. 408-525.

increased variability frequently manifests itself from the first and must be considered as one of the early effects of the foreign sperm.

Can the data derived from remote heterogenic crosses, such as those described by Loeb and Godlewski be safely used as criteria for positing a theory of normal biparental inheritance?

An examination of the work of the two experimenters mentioned and of several other contributions of more recent date, reveals the fact that the spermatozoön in no case functions completely or normally.

In fact, as Loeb himself has suggested, it seems highly probable in crosses between different orders, such as echinoids and crinoids, that the spermatozoön performs only one of its functions, that of initiating development, and that the process of development is thenceforth parthenogenetic.

In Godlewski's experiments it can scarcely be doubted that the sperm nucleus enters the egg and fuses with the egg nucleus. The figures show, however, that the chromatin material remains inactive and that the male pronucleus, instead of increasing in size until it equals that of the female pronucleus, remains in its concentrated condition until it fuses with the latter. This fusion has every appearance of a mechanical absorption of a foreign particle. In no place does Godlewski indicate that the chromatin of the sperm nucleus takes part in the mitotic divisions of cleavage. It operates only to the extent of slightly hindering the rate of early cleavage and probably is soon entirely absorbed by the egg protoplasm.

A still clearer case is that described by Kupelwieser,[5] who fertilized the eggs of an echinoid with the sperm of a mollusc. In this case both description and figures show clearly that the sperm nucleus never breaks up into chromosomes, but remains inactive in the form of a mere lump of inert substance, apparently completely incompatible with the materials of the egg nucleus. In this form it is carried along through several cleavages and is subsequently absorbed. It should not be surprising then to find such hybrids, if hybrids they may be called, showing pure maternal

[5] Arch. f. Entw. Mech., vol. 27, pp. 434-462.

characters. The very fact that there is no sign of a paternal influence should only serve to emphasize the importance of the nucleus as a factor in determining the character of early development.

Bataillon,[6] fertilizing the eggs of several species of Anura with the sperm of the Urodele Triton, obtained results very closely in accord with those of Kupelwieser. There was no real nuclear amphimixis, but the sperm nucleus remains in a mass and soon degenerates.

When individuals belonging to two genera of the same order are crossed there is evidently less incompatability, as a rule. Herbst,[7] for example, has made an extremely careful study of the behavior of the paternal chromatin in the hybrids produced by fertilizing the eggs of Sphærechinus with the sperm of Strongylocentrotus, in which he found that the male chromatin in some cases divides more or less completely into chromosomes and takes part in the mitosis of early cleavage, in others it seems refractory and shows a tendency to go undivided to one motitic pole, and in still others it becomes segregated into a small separate nucleus in the 2-cell stage. Evidently at no period does the male chromatin function normally.

When two species belonging to the same genus are crossed there is sometimes an approach toward complete compatability of the nuclear materials of the two parents. In the two species of Fundulus used in the above experiments we have a case in point. Here there is no visible difference between the chromosomes of the two species and the male chromosomes seem to behave quite normally in cleavage from the first division onward.

One would scarcely be justified, therefore, in drawing conclusions concerning the normal process of heredity from data such as have been described where there is every evidence that the paternal contribution is either eliminated at a very early stage of development or functions in a decidedly abnormal manner.

Is there cytological justification for the statement that "the char-

[6] Archiv. f. Entw. Mech., vol. 28, pp. 43-48.

Archiv. f. Entw. Mech., vol. 27, pp. 266-308.

acteristics of the organism, at least as late as the gastrula stage, are derived entirely from the egg cytoplasm"?

The above statement is based largely upon certain experiments of Godlewski, in which enucleated sea-urchin eggs fertilized with crinoid sperm produced gastrulae of purely urchin type. Examination of Godlewski's records shows that in all these experiments only four eggs developed at all and these did not produce typical larvæ. That this very meager piece of evidence is inadequate and unsatisfactory seems to be the opinion of subsequent workers on echinoderm hybrids. It is certainly not sufficiently well established to form the basis for any important conclusion.

There is undoubtedly a close correlation between the degree of normal functionality of the male nucleus in early development and the degree of hereditary influence exerted by the latter. This was shown in clear fashion by Baltzer,[8] who crossed four species of sea-urchins in all possible ways and noted that when there was a complete elimination of paternal chromatin a pure maternal type of larva resulted, and when the male chromosomes continue to function more or less normally the larvæ showed an admixture of maternal and paternal characters.

The results here discussed seem to point to the conclusion that the nuclear material is the chief factor in determining the character of early development. As Fischel has ably pointed out, *the rôle of the spermatozoön is from the beginning formative in character in that it is able to place the stamp of its own specific characters upon the early developmental stages of the organism, while the egg cytoplasm furnishes only the material for the formative operation of the combined nuclear material of the two parents.*

[8] Zool. Anz. Bd. 35.

STUDIES WITH SUDAN III IN METABOLISM AND INHERITANCE

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INTRODUCTION

In a paper presented before the American Society of Zoölogists, December, 1907, the writer (07) described and demonstrated, among other things, the deposit of Sudan III in the egg of the domestic fowl. A very brief abstract of this paper was published in Science, June, 1908. All of the data concerning the method of introducing the color into the eggs, and the significance of this in metabolism and inheritance, as well as the data concerning an interpretation of the white and yellow yolk of vertebrate ova, were then written up together. It now seems advisable however, to treat the first two subjects apart from the last since the phenomena

of inheritance involved in the transmission of this aniline dye have attracted an amount of attention which make a fuller treatment necessary.[1]

It is important that any statement concerning the *inheritance* of this or another pigment be accompanied by a statement of the facts concerning the behavior of this substance in *metabolism.* That is to say, if we make statements concerning the passage of a substance through follicular cells and of its deposit, distribution, behavior or development within germ cells and in their derivatives, it is obviously important to keep in view what we know regarding the way this substance passes through other membranes; how it exists, is distributed, behaves or develops in somatic cells. It is of value to realize *how* these things happen in any membrane or cell of the body, because it is this same how, or in other words, the *mechanism* of such successive transformations in the germ, that is the very *self* of inheritance. Again, if we state our results on inheritance in terms of metabolism we are, in this case at least, less liable to exaggerate the importance of our

[1] During the two years since this paper was prepared and read other workers have undertaken and reported work along similar or related lines; and quite recently, within four months, three papers have appeared which make it seem advisable to divide this paper into two parts, and to publish in full without further delay the part most intimately connected with the present title. One is the more readily persuaded to this division and immediate publication because of a communication which appeared in Science, September, 1909. Dr. Ludwig Sitowski there directs attention to the fact that he had secured the deposit of Sudan III in the eggs of moths, and had published an account of his experiments as early as 1905, but that his paper has been overlooked until now by the writer and apparently by others. With pleasure the writer hastens to make acknowledgment of his excellent work. Only the timely appearance of his communication together with the fortunate circumstance of delay in the publication of this article have made it possible to give his work the credit it deserves. It is hoped that Dr. Sitowski will realize that the place and title of his publication were such as to make it not difficult for American workers to overlook.

Sitowski evidently has not seen even the abstract of the writer's paper but only a short notice or review of it which was written by H. A. in the Zeitschrift f. d. Ausbau der Entwicklungslehre, Bd. III, Heft 2, 1909.

The writer's report of this work has received notice in several quarters, and in almost every instance he has been credited with the works of others, or to others has been attributed work done by him. In these shiftings he usually has fared better than he deserves; but it seems that the results thus far obtained in this field should be brought together in such a way that it may be made plain just what has been done and incidentally who did the work. It is partly for these reasons that the entire literature on Sudan, so far as the writer has been able to find it, is here brought together and an outline of the results given.

results; while at the same time we get a close view of the sort of mechanics which is back of this type of "inheritance." We may state at once our belief that we are here dealing with a noteworthy, though very simple, form of inheritance, but one which seems by no means sufficient to illuminate hereditary processes in general.

By the use of Sudan the writer has been able to demonstrate some hitherto unrecognized features of fat metabolism, particularly as it occurs in birds. It seems both convenient and helpful to report these results in connection with the present survey of Sudan in metabolism and inheritance.

HISTORICAL

The historical background of the present studies is furnished by a view of the state and source of our knowledge of the four following topics:

(1) The living membranes through which Sudan will pass, and the points at which it is deposited in the body.

(2) The mechanism of the transfer and deposition of Sudan within the body.

(3) The uses to which Sudan has been put in experimental biology and medicine.

(4) Sudan III, and other pigments, in "inheritance."

In summarizing the data on these topics it has been considered to the advantage of the reader, to include the results of the writer as well as those of other investigators whose findings have been published since the preliminary report of his own work. The summaries, therefore, are believed to be complete to date.

(1) *Membranes Through which Sudan is Known to Pass*

Intestinal mucosa: birds and mammals, (Daddi, '96).
Intestinal mucosa: human, (Franz and Stejskal, '02).
Intestinal mucosa: moths, (Sitowski, '05).
Embryonic intestine: yolk-sac of chick, (S. H. & S. P. Gage,'08).
Renal epithelium: human, (Franz and Stejskal, '02).
Epithelium of egg chamber: moths, (Sitowski, '05).

Follicular epithelium: birds, reptiles, mammals, (Riddle,'07 b).
Epithelium of mammary glands: rat, (Gage, Stotsenberg,'08 b).
Thecæ of Corpus luteum: rabbits, (Riddle, this paper).
Peritoneum: birds, (Riddle, this paper).

(2) *The Mechanism of the Transfer and Deposition of Sudan within the Body*

Practically all we know concerning the mechanism of the transfer and deposition of Sudan we owe to the feeding experiments and observations of Daddi, to the studies on the solubility of the stain by Pflüger and Nerking, and to the chemical researches of Michaelis.

Daddi ('96) found that when fed to rabbits, guinea-pigs, pigeons and fowls, Sudan passes through the intestinal epithelium, enters the circulation and is deposited in the adipose tissue of the body generally; that it is a specific fat strain, and that in its introduction and deposition in the body it is always associated with fat. Biedermann, ('98) was the next to use Sudan experimentally. He fed the stain to Tenebrio molitor and found that although the intestinal contents became colored, the body fat was not colored.[2] Hofbauer ('00) made the mistake of supposing that only natural fat and not soap and fatty acids, was able to carry the dye through the intestinal epithelium, (he used Alkanna which resembles Sudan in its solubilities, and he also refers to Sudan). He thought, therefore, that by use of the stain he could determine in which form the fats are absorbed from the intestine. The paper containing this error, two other papers which repeat it, together with the three or four papers devoted to exposing the error, furnish nearly one-half of the literature dealing with Sudan III. Pflüger ('00) and Friedenthal ('00) simultaneously pointed out Hofbauer's mistake, showing that under the conditions furnished by the intestine Sudan is soluble not only in fat but in bile, in sodium soap and in glycerine. Friedenthal, however, declared that soaps have no power to dissolve the stain in the absence of free fatty acids. Nerking ('00) immediately showed that entirely

[2] Prof T. H. Morgan informed the writer that in a fly, Drosophila, he has recently obtained a similar result.

neutral soaps do have the power to dissolve Alkanna, Lackroth and Sudan. It may be pointed out that Sitowski ('05) repeated Hofbauer's error although he based no conclusions upon it. Whitehead ('09) without knowing of Hofbauer's work attempted to solve the same problem in a like manner. Mendel ('09) has shown that Whitehead was further mistaken in his observation that after a dog has been fed with Sudan, the lymphatics of his mesentery remain unstained. It may be stated here that there is no difficulty whatever in finding the stain in the lymphatics of fowls, if these be examined two or more hours after being fed the dye.

Michaelis ('01) made very careful chemical studies of Sudan and related compounds. He considers the staining of fat a physical and not a chemical process, and draws the conclusion from his work that the physical properties of a body depend upon its chemical character since the dye molecules, to be soluble in fat, must have a very definite constitution. He concludes, "fat will be stained by those azo-dyes which are 'indifferent' in the sense of possessing no salt-forming groups." Mann ('02) has given some consideration to the nature of the union of Sudan with fatty compounds, and on the basis of Michaelis' studies, states (p. 310) that "the union between Sudan III and oleic acid is a chemical one depending on the oxidation of the unsaturated fatty compound. Therefore, the action of Sudan III and similar dyes is analogous to that of osmium tetroxide, the only difference being that azo-dyes form additive compounds with the fat without loss of color, while osmium tetroxide, after having formed additive compounds, is readily decomposed owing to the high valency of the osmium." From either view-point, or indeed from any possible viewpoint, it seems certain that the dye is bound to the fatty constituents, cannot loosen from them, and is dragged with them mechanically, so to speak, wherever they may go.

(3) *The Uses to which Sudan has been applied in Experimental Biology and Medicine*

As has been noted, Daddi introduced the stain into histological work, used it as an intra-vitam stain, and by its means studied the foci and extent of various fatty degenerations of the liver

and muscles. The futile attempts of Hofbauer, Sitowski and Whitehead to determine the form in which fats are absorbed from the alimentary tract have also been mentioned. Franz and von Stejskal ('02) made extended studies on the fat metabolism of a chyluric by means of the stain. When stained fat was fed to the patient the colored fat appeared in the urine in four hours and continued always less than twenty-four hours. When, however, stained fat was injected subcutaneously into the shoulder it did not appear in the urine.

Sitowski with this stain undertook the solution of certain problems of digestion in the caterpillar of certain moths. He made little progress with these problems, but in the course of his investigations discovered a deposit of the dye in the eggs (primary oöcytes) of these forms. The writer has used Sudan in a number of studies and for several purposes. He will mention at this point merely its use in determining the *time* and *rate* of growth of the eggs (primary oöcytes) of fowls and turtles; in the study of some special features of fat metabolism; and to gain some information as to the inter-relation of the soma and germ cells, including transmission and inheritance behavior. Gage has followed the first report of our results with further studies on the behavior and distribution of this dye in the developing fowl, its appearance in the milk, and with negative results, its passage through the placenta.

(4) *Sudan III and Other Pigments in Inheritance*

The only recorded cases known to the writer, of the deposit and persistance of foreign or maternal pigments within germ cells, are the following: Schmidt ('91) found that Alkanna-colored fat was taken up by apparently all plant cells; the ovules of these plants are not specifically mentioned as obtaining part of this coloring matter.[3] Pizon ('01) states (p. 170) that "the first pigment of the larva (Botryllides) proceeds from the maternal organism by migration." His observations are not conclusive. Sitowski ('05) fed Sudan to caterpillars and obtained the stain within their eggs. He now reports ('09) the presence of the stain

[3] Fat colored with Alcannin had been used by Pfeffer (Osmotische Untersuchungen, 1877) to color the fat being injested by Myxomycete plasmodia.

in the somatic structures of larvae hatched from such eggs. The writer ('07) caused birds and turtles to deposit quantities of Sudan within their eggs. S. H. and S. P. Gage ('08) have hatched Sudan-containing eggs of the fowl and noted the re-distribution of the stain in the somatic tissues. In addition to these cases, however, it should be noted that it is practically certain that the natural coloring matter of the eggs of the salmon proceeds from the muscles of the fish. It is quite certain that the *fat* which is in these muscles, and in which much if not all of the coloring matter resides, is transferred to the ovary and to the growing eggs. These lipochromes of the muscle fat doubtless remain fixed to the constituent fatty acids, when this fat is broken up in the muscles and is thrown into the circulation; from the blood or lymph, we believe, the two enter the ovum together, precisely as in the case of Sudan-stained fat.

EXPERIMENTAL METHODS

Laying hens were fed Sudan III in three ways, viz.: in gelatine capsules, dissolved in egg-yolk, butter or animal fat, or by enclosing small lumps of the stain in pieces of bread (no fat). The results were very similar in all cases. With birds, the method of feeding seems quite immaterial since the stain apparently always meets with enough fats within the alimentary canal to carry considerable quantities of it through the intestinal wall. The dose varies from one-half gram to three milligrams. For most studies, particularly those dealing with problems of metabolism, large doses are to be avoided; from three to twenty milligrams have been found most useful, Many birds were fed the stain at intervals of thirty-six, forty-eight and seventy-two hours; series of eggs from birds thus fed were obtained, were hardboiled, sectioned under water with a sharp razor and then examined, these latter operations being done chiefly to learn the rate of growth of the ova. In other cases the birds were killed at such time after feeding as was demanded by the points under investigation.

The stain was introduced into the bodies of chicks and rabbits also by injection of its solution in a mixture of oleic acid and alcohol. A widely variable quantity of the solution was injected

into the peritoneal cavities of these animals (also into brachial veins of the chick, and ear veins of the rabbit). The method of feeding the stain to turtles will be described with the results of that work.

RESULTS OF THE EXPERIMENTS

Deposition of Sudan III in Ova

The eggs of hens fed as described above almost invariably showed marked quantities of the pigment deposited in the yolks. An ovum which had undergone its final and rapid growth in a bird which was being fed at regular intervals of thirty-six, forty-eight or seventy-two hours would show in section a series of evenly-spaced, concentric circles of orange-red, these alternating with other circles of light yellow, the natural ground color of the yolk. The width of any Sudan-colored circle could have been regulated at will at the time of feeding; much stain giving the wide rings of red, and little stain resulting in narrower rings of less intense color. If the birds be killed a few hours after feeding all of the larger ova are found to be deep red on the outside; if, however, the bird be not killed until one or two days have elapsed since the feeding, these ova will have a perfectly normal external appearance, and only an examination of the interior of the eggs will reveal the presence of the stain. This is, of course, a consequence of the rapid growth of these ova.

One successful attempt was made to stain the ova with Sudan injected into the peritoneal cavity. Four injections of a mixture of alcohol and oleic acid forty per cent each, to which traces of sodium carbonate were added, were given within a period of forty-eight hours. Four eggs were subsequently laid by this bird and were found to contain the dye.

In the case of the turtles the records are as follows: Three very large females of Emydoidea blandingii were heavily fed with Sudan for three weeks during July and August. The stain was put with butter into capsules of large size and these were pushed with long-slender forceps into the stomach, while the neck was stretched and the mouth held open with other forceps. All were killed five days after the last feeding. All of the larger ova

showed the characteristic color of Sudan at their peripheries. The thin, follicular membranes were slightly tinged with red. In January four similar turtles were fed and killed in the same manner. In none of these cases could Sudan be found *within* the eggs, although some of the follicular membranes seemed very faintly stained.

This different result during the two seasons is of considerable interest from the standpoint of determining the season in which the eggs of turtles grow. If the eggs had been growing (depositing yolk) in January, they should have taken up the stain. The fact that they failed to do so is evidence that they are not growing in January. The definiteness of this finding is somewhat vitiated however, by the writer's observation (09) that the digestive capacity of these forms is very low in midwinter; and by the further observation that the forms which were fed the Sudan in winter sometimes regurgitated parts of it. It cannot be stated as certain, therefore, that as much Sudan was put into the *blood* of the turtles in winter as in summer. (The turtles were kept from summer until January in aquaria containing water at outside temperature. At the beginning of the feeding experiment they were brought into water at summer temperature, about 20°).

The ovaries from rabbits injected (with the same solution as for the birds) once or twice daily for a week, were examined. Only two of these animals survived the injections long enough to be considered seriously. One of them showed no certain traces of the stain anywhere in the ovaries, the other, only in the corpora lutea. This work on the rabbits was shared by Prof. S. A. Matthews.

Deposition of Sudan III in the Soma

Fowls heavily fed on Sudan, for even a day or two, usually show upon examination a reddish color in all their adipose tissues, most prominently in subcutaneous and peritoneal fat. This but confirms Daddi. In addition to his findings, however, it was determined that if newly hatched chicks be fed the stain during the growth of the juvenile plumage the feathers also take up the stain and become distinctly red in color; (the Sudan-containing offal was often and completely removed from the brooders and pens to prevent its be-

ing mechanically scattered over the outside of the plumage). The claws and bills of the birds likewise become highly colored, but one cannot be perfectly certain that this color is not of external origin. Injection of the stain gave very similar results; in these cases, however, a more diffuse color was obtained, no attempt was made to color the feathers and no staining of the intestinal wall was noted. These birds laid down colored fat after having been given the stain by injection into the peritoneal cavity.

After feeding the stain to turtles one finds but traces of Sudan deposited in somatic tissues. This is undoubtedly due to the fact that they *store* fat extremely slowly, and that their bodies actually contain but little fat. The further fact of the difficulty or slowness of digestion which the writer (09) has found especially to characterize the turtles, may also be important in this connection.

The subcutaneous fat and the intestinal mucosa were the only parts other than the ova and follicular membranes in which the writer found the stain deposited in turtles . No injection of the dye was attempted in these animals.

Rabbits apparently ingest Sudan much more slowly than do fowls. Nevertheless, upon continuous feeding red-colored fat becomes visible in all parts of their bodies, subcutaneous fat everywhere, peritoneal and kidney fat, the intestinal wall and corpora lutea. Daddi noted a similar distribution (except in the corpora lutea) in rabbits and guinea-pigs. A similar distribution of color results from the injection of the stain (except for the mucosa).

The Rate and Conditions of Absorption and Deposition of Sudan III

Almost no attention has been given by previous writers to the rate at which Sudan is absorbed and desposited. Since this really represents the rate at which *fat* is absorbed and deposited, it becomes a matter of considerable interest. Similarly, the conditions of its deposition and non-deposition when it is brought within the blood-stream, have nowhere received consideration, except, of course, that it has been generally noted that it is deposited in fats. The writer is able to report approximately correct data on these points as they were obtained in the study of the fowl only.

Many birds were killed soon after feeding with Sudan; the time intervening between feeding and killing ranging from one-half hour to days and months. From the examination of these birds it was learned: (1) That the stain may appear in the mesenteric lymphatics within a period of seventy minutes after feeding; (2) at end of two to three hours after heavy feeding a perceptible amount of stain is laid down in the rapidly growing ova; (3) the body fat becomes colored much more slowly than the yolk fat; (4) the several regions of body fat are not all colored simultaneously, even the subcutaneous fat of some regions remaining colorless at a time when subcutaneous fat elsewhere is quite red. This last fact seems to indicate that there are differences between these several "storehouses" of fat; that some are centers of a most active commerce, there being in these a continuous loading and unloading of wares; whereas there are other storehouses of fat whose portals during normal conditions at least, are quite tightly closed.

In regard to the conditions under which Sudan is, or can be, deposited, we have determined the following facts: (1) Sudan can be deposited only in *growing* ova. Indeed, for a perceptible amount of the stain to be taken up the ova must grow more rapidly than do those ova of the fowl which are less than 5 mm. in diameter. (2) Sudan can be deposited with difficulty, and only in small amounts, in a fowl that is not being fed and is thus made to use its store of fat instead of being allowed to grow new fat. These results have been verified on so many birds that there is no doubt of their being entirely reliable. It cannot be said that the starved animals did not get the fat into their circulation because of failure to absorb the stain under the starving conditions, for some of these birds were given the stain by injection, and they too showed just as decidedly the results stated above. One cannot but see in these two results the very strongest evidence that *while in the body, Sudan III clings at all times to the fats or their constituent fatty acids, and so goes quite mechanically wherever these particles go; it is indeed, attached to them.* (3) There is moreover in lightly-colored fat a marked tendency of the stain to remain in this fat in the living animal and not to leave it for other contiguous fat. This was shown by the sharpness of the inner edges of the bands of stain in the ova, as well as by one's ability to circulate stain through the

body of animals not depositing fat, without coloring certain regions of fat. (4) It was found that within the ovum the Sudan is deposited in the germinal disc and in the latebra in smaller amounts than elsewhere. This is undoubtedly to be associated with the lower fat content of these regions of the egg.

AN UNSUSPECTED ACTION OF SUDAN

An Apparent Tendency of Sudan to Lessen the Availability of Fats in the Organism. Since 1904, when the writer first fed Sudan to chicks, several things have come under his observation which indicate that *Sudan-stained fat is not as available,—does not split up and yield its energy to the organism as readily—as does the unstained fat.* If this could be positively established it would be a very important fact, possibly giving some clue as to what "availability" of foods rests upon. We might, perhaps, then proceed so to treat certain foods or constitutents of the tissues as to increase or decrease at will their utilization or destruction within the body.

Some special effort has been made to get positive data on this hitherto unsuspected action of Sudan. It must be admitted that conclusive data have not been obtained; in their absence the writer can only submit the following record of efforts,—a few observations and experiments which seem to contain some bits of evidence:

Biological Evidence

(1) Young chicks which were given Sudan with their food ate much more than those not fed the stain; they seemed always hungry and did not grow as well as the other birds of the same age and breed. This, of course, may easily have another explanation than the one suggested.

(2) In a certain "starving" experiment it happened that birds three months old were used, five of which had been given three heavy feedings of Sudan during the two days immediately preceding the starving period. These five Sudan-fed birds were all dead before any of the four non-fed ones showed very great signs of weakness. Three of the five dead birds were carefully examined. They showed Sudan in patches of subcutaneous fat, in other patches along the neck, behind the occiput and even distinct traces

in peritoneal fat. On the other hand, the muscles showed extreme waste. Two of the birds from the other pen were now sacrificed for comparison. They showed hardly a trace of fat. Dipping them into an eighty per cent alcoholic solution of Sudan failed to reveal more than traces. The muscles, however, were obvioulsy larger and much better preserved than in any of the Sudan-fed birds. Sudan was found to be a non-toxicant as many birds were fed several months and one adult hen was fed the stain almost continously for ten months without visible injurious effect. This fact, together with those mentioned above, lead one to suspect that the presence of the stain in the fat made this fat in these birds less available than if unstained, and that under the new conditions the energy of the proteins (of the muscle, etc.) became more available than that of the fats.

(3) If birds be fed considerable quantities of Sudan while growing a plumage it will be found that the "fault-bars"[4] of the feathers become more pronounced in extent. It has been shown conclusively that any decrease in the nutrition of the feather germs produces these effects. Attention has elsewhere (Riddle, '07[a] p. 172) been specifically called to this power of Sudan to produce fault-bars or defective areas in feathers and to the fact that this seems to be due to a starving effect produced by the Sudan.

(4) It has also been pointed out by the writer (08, p. 174) that if young chicks in their downy plumage be "starved" for a time, or fed Sudan in quantities, there is a common result in the two cases, namely, an inhibition of the growth of most of the definitive feathers and a long retention of the downy plumage. This is evidence of the sort we are just now examining, since these Sudan effects so closely parallel "starving" effects. The following case is perhaps less valuable evidence of the same kind.

(5) It has been observed that many laying hens cease to lay eggs after having been fed considerable quantities of the stain. The effect here is again the same as that resulting from a withdrawal of food; it may, however, have other causes as well.

(6) The above and similar observations led to the following experiments. The one here recorded was made after nos. 7 and 8

[4] See Oscar Riddle on the Genesis of Fault-bars in Feathers and the Cause of Alternation of Light and Dark Fundamental Bars. Biol. Bull., vol. 14, pp. 328-370, 1908.

had failed to satisfy. Six plymouth rock hens in good condition, not laying, were isolated for the experiment. Quite at random three of these hens were taken and given food + Sudan capsules for two days; the other three were given food but no Sudan. The birds were then all removed to a pen where they could get no trace of food; only water was given them. The weight of each bird was taken at the beginning and on each third day of the experiment during its fifteen days of duration; the object of all this being to learn which group of birds would lose weight faster. It was thought that those using most fat would lose least weight and vice versa; viz., that energy must be supplied to the birds during life, that if they secure this energy from the fats of their bodies instead of from their protein, they will need to use fewer grams to obtain any desired amount of energy since the energy content of fats is to that of proteins as about 9.3 to 4.1. An important part of the records of the weighings unfortunately has been misplaced and the writer cannot give the exact figures; but *the net result showed that each of the Sudan-fed birds had lost, at the end of the period, a higher percentage of its initial weight than had either of the non-fed birds.*

Chemical Evidence

(7) It was thought that if the stained fat were less available to the organism, as seems to be the case, this might be connected with a decreased power of the fat-splitting ferment to split such fat. The following attempt was made to determine this point; lipase was prepared from the castor bean and equal quantities of this was put into flasks, one set containing oil +Sudan, the other pure oil only. Flasks of the two sets, left on the shaking machine and given time for hydrolysis, were then titrated with n. 10 Na O H, and compared. It was found, however, that the strong and persistent color of the Sudan so obscured the expression of the indicators that it was quite impossible to determine the neutral point in the Sudan-containing flasks. The titration method of estimating the rate of hydrolysis of the fat, therefore, had to be given up. An attempt was next made to determine the rate or amount of digestion in the two sets of flasks by measurements of their elec-

trical conductivities. This proved impracticable because of the extremely low conductivity of the oils. The writer is therefore not prepared to state whether the presence of the stain in the molecule of fat has any effect upon the power of lipase to hydrolize that molecule.

(8) It seemed advisable next to learn the effect of the presence of the stain on the rate of the spontaneous oxidation of the fats. By a method described elsewhere[5] Prof. A. P. Mathews and the writer, in connection with other work, made a few experiments on the rate of oxidation of linseed oil with and without the stain. It was determined that the oxidation proceeds more slowly in the oil + Sudan than in pure oil. Light has, however, such a profound effect upon the rate of oxidation that it is perhaps possible to attribute much or all of the retardation measured in our experiments to the absorption of light rays by the Sudan. The question that has been raised of the lessened availability of Sudan-stained fats must then be left without conclusive answer, but with such evidence as the preceding statements afford.

DISCUSSION AND CRITIQUE

The main facts at hand have already been given in a rather long historical statement and in the preceding account of the writer's own results. The specific statements on the several topics of fat metabolism need not be again referred to. The general question of the basis or source of usefulness of Sudan III in such studies as the present may, however, be touched upon here. We can now consider too another most interesting aspect of our subject, namely, the significance in inheritance of the observed transmission of this aniline dye from soma to germ cell, and its redistribution among the daughter cells of the germ. We treat the former topic first.

In the study of the problems of fat metabolism, what is it that gives value and significance to the use of Sudan III? The answer must be that it is because Sudan sticks to fat or fatty constituents as long as they remain such in the body. Where the

[5] See article by A. P. Mathews, O. Riddle and S. Walker, The Spontaneous Oxidation of Some Cell Constituents, Abstract in Journ. Biol. Chem. vol., 4, p. xx, June, 1908..

original[6] stained fat goes, we believe from our experience, the stain will go also; the tell-tale color of the Sudan betraying at once both the *presence* and the *source* of the fatty materials in transformation. We are thus enabled to study such aspects of fat metabolism as involve transfer, and re-deposition of fat, etc., which have been open to almost no other means of attack. Indeed, few organic constituents of the body other than the fats, are open to such methods of study even now. The sum of our present information shows quite clearly that the Sudan holds to the constituent fatty acids even when the integrity of the fat molecule is lost; this, during its transfer within the body fluids, through practically all of the membranes of the body, and during re-synthesis, in whatever part of the body this may occur. In all these states and relations the pigment maintains the union; apparently only during the oxidation and final destruction of the fat is the alliance broken. How far the oxidation must proceed before the disunion occurs, the writer is unable to say.

The facts already brought forward concerning the behavior of Sudan in several aspects of fat metabolism furnish some solid ground upon which to base a discussion of the transmission and "inheritance" phenomena involved in the passage of Sudan into the egg and the embryo. We can get a clear vision of this field of fact if we now focus on two points: What are the processes concerned in the entrance of the dye into the egg, and in its re-distribution in the newly arising cells of the embryo? How do these processes compare and contrast with processes known to be involved in inheritance and developmental phenomena? The answer to these questions should bring into relief a safe estimate of the significance of the transmission phenomena in question.

The facts absolutely support the view that the passage of the stain through the follicular membrane, which has here been shown, is in no way unlike its passage through the intestinal epithelium or any other membrane. The Sudan, playing here an entirely passive rôle, is taken mechanically to whatever point the fat goes and remains with the fatty acids wherever they again become anchored through resynthesis into fat. The processes involved

[6] This holds true apparently when the fat is *lightly* stained. Statements made elsewhere furnish the necessary qualifications, and the evidence.

in the re-distribution of the stain and fat in the cells which arise by division of the egg, are not different. Here we must believe that each cell of a dividing pair will carry stain in very close proportion as it carries fat. This is the testimony obtained from all somatic tissues and the writer has shown that the general conditions of this statement are fulfilled in the oöcyte and egg itself, since the germinal disc and the latebra of these stages take least stain (intra vitam) and are known to contain least fat. When in the course of development there arises a variety of body regions, some of which are less favorable for oxidations and therefore more favorable forthe storage of fats, the stain-containing fats may become transferred to these regions of the embryo, precisely as occurs in the somatic tissues of the adult. Localized areas of stained fat thus arise during embryonic life.

If now one compares and contrasts these processes with those known to accompany inheritance, i. e., developmental processes, some interesting features appear. There is, to be sure, *transmission* of the dye from soma to germ, there is a *persistence* of that which is transmitted to such an extent as to cause this soma obviously to display the "new character." If in the chick the body fat were used up in egg production,[7] as was elsewhere noted to occur in the salmon, some of the dye would of necessity again be deposited in the several eggs next formed; these eggs would in turn supply the somatic tissues developing from them. *But this must inevitably come to an end in a few generations*, the stain, sooner or later, having become diluted to the vanishing point. Again, *there is absolutely no new growth* of the material forming this "character," nor is there any *chemical change* either in early or in late phases of the life cycle. *Morphological change* does however, accompany each change in the disposition of fat within the organism, the color-picture thus being a moving one, *different in each succeeding stage of development.*

These striking contrasts with what we recognize as the basic things in developmental phenomena may well cause many to inquire: Why do we stop at all to consider the phenomena under

[7] I have observed a hen to lay four eggs after the beginning of a "starvation" experiment; the last of those eggs was laid on the twelvth day of starvation and much of the fat of its yolk was undoubtedly derived from the body fat of the bird.

observation, as inheritance phenomena? The reason is that inquiry and reflection seem to attest that this behavior of aniline dye is not an isolated thing in nature but that certain behaviors are known which are universally treated as "hereditary," and which rest upon essentially the same base. There is then, a group of cases which exhibit the simplest known inheritance phenomena, and which may be considered in the light of, and be largely explained by our experience with Sudan.

At the outset we call attention to the simplest analogy: the fact that the entire fat content of the egg yolk, which in the egg of a fowl aggregates several grams, is without doubt transmitted from the soma to the egg in the same way that Sudan is transmitted. That is to say, the fatty acids, which are re-synthesized into fat within the yolk pass from the soma (i. e., from within the body fluids) through the follicular membrane as these same fatty acids; the fatty acid constitutents of the egg-yolk by no means originating *within* the egg. This conclusion follows as a logical necessity from our knowledge of fat metabolism elsewhere in the body, as well as from the special findings of Henriques and Hansen (03), who report the recovery of the specific and foreign fats of the food from the egg-yolk of the fowl. Moreover, from the standpoint of our general knowledge of metabolism it is not to be expected that these constitutents of the egg-yolk should reach the latter in any other form. The protein of the egg-yolk, must also be conceived as having entered the egg, or at least to have approached it, in a simpler form than protein, namely, as amino acids, etc.; the reconstruction of these doubtless occur chiefly within the egg itself and in this way give rise to the complex and special proteins of the egg.

These things which occur in the formation of every egg, these "transmissions" of amino and fatty acids from soma to germ, are cited because some biologists have considered the passage of a molecule of dye, (azo-benzene "azo" β naphthol) from soma to germ, a thing not at all to be expected. Perhaps this state of thought is but an echo of the thoroughness of our long instruction on the wide gulf supposed to separate germplasm and somatoplasm; on an implied immunity proceeding from follicular walls, and an inviolate incorruptibility thought to preside over

all that lies within a vitelline membrane. Nevertheless the analogy holds: the mass of fat and protein in the egg of the fowl is transmitted from parent to germ. This fat, moreover, neither immediately disappears nor undergoes equal distribution in the embryo, but like the Sudan it *persists* and becomes *specifically localized*.

As an interesting example of such localized persistence the writer cites the toad's egg in which Miss King (08) has pointed out that *masses of yolk from the developing egg persist in the new germ cells*, and that the yolk masses serve to mark off these germ cells as such. Here occurs a passing over of certain constituents from one germ cell to the next generation of germ cells. Of course it cannot be asserted positively in this case that the identical fat molecules of the first egg were contained in those of the succeeding generation. Miss King merely asserts the continuity and persistence of the morphological picture furnished by aggregates of such molecules. The transmitted fats however, exhibit one more advanced stage of complexity of behavior than does Sudan, due to the new combinations they can enter into and the readiness with which their molecules can be both built up and torn down in the organism.

A second analogy of the *transmission* and *temporary persistence* of Sudan we find in the cases of hereditary immunity, observed hitherto chiefly in mammals. In these cases, as is becoming well known, the immunity secured by the fœtus through the placenta or germ may be of longer or shorter duration, often covering only a fraction of the span of a single generation. It is from the standpoint of the type of transmission displayed by Sudan that these inherited immunities are to be interpreted.

Sitowski has given the analogy of the passage of parasites (spirochætæ and other protozoa) from the soma into the reproductive cells; the analogy is not complete, as he has pointed out, since in the case of the parasites we deal with living, active forms which seek out the germ cells. Other differences might well be noted. Bacteria also are known to reach germ cells in a similar way.

Of much more interest and weight is the analogy between the behavior of Sudan and that of the glow substances of the glow-

worm. In Lampyris and Pyrophorus these substances are to be found in the eggs and in every intervening stage up to the adult. It is true that in this case the substances, in contrast to the Sudan although not contrasting with the fat, do increase in amount at given points in the cycle. But the general features of the two cases agree so closely that actual kinship of behavior seems certain.

In conclusion, we may emphasize the fact that the transmission behavior of Sudan is the simplest of a simple class, and one of which we can render mechanical explanation at practically every point. It shows to us the simplest form of inheritance, if the above analogies be granted. If the entrance of fat into the egg and its persistence there is as stated; if the hereditary immunities are of similar origin; or if the "glow" susbtances mentioned owe any considerable amount of their persistance to the type of mechanics with which we have been dealing in the case of Sudan, then this latter substance has proved of value in giving a view detailed and clearcut, of the mechanism of some phenomena which have been generally considered inheritance phenomena. The writer does not forget, however, the striking contrasts which these cases present to the great bulk of developmental phenomena, and which *seem* to present quite a different magnitude of complexity. He wishes to acknowledge his inability, for the present at least, to state how the simpler cases here considered are to enter very deeply into a solution of the more complex ones.

It need hardly be pointed out, after our detailed account of the action of Sudan, that these studies furnish no basis whatever for the inheritance of acquired somatic characters.

SUMMARY

1 Sudan III fed to fowls and turtles is deposited in their growing ova.

2 Ova and soma of birds and mammals take up this stain after injection into the circulation or peritonial cavity.

3 The dye molecule is closely united with the constituents of the fat molecules and does not usually separate from them in the body.

4 Evidence is obtained indicating that the fat of certain regions

(in fowls) may increase actively in amount whilst other regions of fat take up no new molecules of fat whatever.

5 The stained fat may appear in the mesenteric lymphatics as soon as seventy minutes after feeding. Perceptible amounts may be deposited on the periphery of growing ova one or two hours later.

6 The stain is taken up very slowly, or hardly at all by birds which are being starved and thus made to decrease their store of fat.

7 Fat stained with Sudan is apparently less available to the organism than is unstained fat.

8 The stain which is passed through the follicular epithelium into the egg, i. e., into the newly arising organism—shows there a selective distribution; least stain being found in those parts of the egg which contain least fat, namely, the germinal disc and latebra.

9 The significance in inheritance of our experience with Sudan lies: (1) in the fact that here we get—through relatively accurate knowledge of the properties and physiological behavior of this aniline dye—a clear picture of how particles of the food or soma become a part of the germ or new generation; (2) in the emphasis which it lays upon the fact that the normal constituents of the egg have a comparable history; (3) in the seemingly perfect parallel which it offers in explanation of the inheritance of immunity, etc.; (4) and the possible light which this extremely simple form of inheritance may throw upon the mass of developmental and inheritance phenomena which seem to be of a much higher order of complexity.

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THE EFFECT OF SELECTION UPON MENDELIAN CHARACTERS MANIFESTED IN ONE SEX ONLY[1]

W. E. CASTLE

In the Journal of Experimental Zoölogy, vol 7, No. 4, Miss MCracken reports extensive and important observations upon the inheritance of the alternative race-characters of silk-moths, univoltinism (one generation a year) and bivoltinism (two generations a year). The inheritance she characterizes as non-Mendelian on the ground (1) that when a cross is made involving the contrasted race-characters neither condition appears to be uniformly dominant and (2) that in subsequent generations neither condition can be wholly freed from the other, that is neither behaves as an extracted Mendelian recessive, and (3) that the proportions of univoltins to bivoltins following a cross does not approximate an ordinary Mendelian ratio, but changes from generation to generation according as selection is made for one condition or the other.

It seems to me, however, that these reasons are not sufficient to establish the non-Mendelian character of the inheritance, but on the contrary are entirely consistent with a Mendelian interpretation. In the first place it is to be observed that the inheritance is strictly alternative. All broods are either bivoltin or univoltin in character. This is *prima facie* evidence in favor of a Mendelian interpretation. The two essential features of Mendelian inheritance, dominance and segregation, are both strongly in evidence throughout the entire experiment. The only obscure points from a Mendelian standpoint are these: (1) Is dominance reversed within the series, and (2) are the ratios obtained Mendelian ratios.

Confusion in the interpretation arises fron the fact that the univoltin or bivoltin condition is manifested only in the female line, though transmitted through both sexes. The female silk-moth hatched in the spring of the year lays a batch of eggs and

[1] Contributions from the Laboratory of Genetics, Bussey Institution, Harvard University, No. 5.

then dies. These eggs either hatch and produce a second generation of moths the same season (bivoltinism) or else hold over to the next spring before hatching (univoltinism). All the eggs laid by the same moth behave in the same way, regardless of the character of the male that fertilized the eggs, as well as of the character of the moths which are to develop from the eggs. Thus a univoltin mother may produce both univoltin and bivoltin daughters, but neither sort will hatch from the egg before the following spring. And the eggs of a bivoltin (spring generation) mother will all hatch the same summer, regardless of whether her female descendants are to function as bivoltin or univoltin egg-layers.

It therefore becomes somewhat difficult to trace the decent of the contrasted race characters. And to free either condition from the other, when they have once been crossed, is doubly difficult because the germinal constitution of the individual can be detected neither in the adult males, nor in the second (summer) generation of females.

Plant-breeders have encountered puzzling conditions of a somewhat similar nature, but happily have found a complete and simple explanation of them. This explanation, I believe, will apply with modifications to the case under discussion.

In maize, red color of the seed-coat (or pericarp) is a Mendelian dominant to its absence (white seed-coat), but the red color of the seed-coat is of purely maternal origin, and has no relation to the transmission of red or its opposite by the embryo lying within the seed-coat. If the plant of red-seeded maize is pollinated with pollen from a white-seeded variety, red seed is produced though the contained embryos are heterozygous, red (white). Now if this seed is planted, again only red seed will be produced, the heterozygous mother plants showing only the dominant character in the ears which they bear. But the embryos contained within the second crop of seed will be of three sorts in accordance with Mendel's law, viz: 1RR, 2RW, and 1WW. Plants raised from embryos of the first two sorts will bear red ears, but a plant raised from a WW embryo will bear white ears. even though the seed-coat which covered that embryo was red, And if such WW plants are self-pollinated or pollinated *interse*, no red ears will be obtained thereafter, as shown by Locke ('06).

The behavior of red pericarp color is throughout this experiment consistently that of a Mendelian dominant, the only peculiarity of the case being that the dominant character is manifested only in maternal structures, not in paternal ones or in those of the embryo itself.

Again, if white-seeded maize is pollinated with pollen from a red-seeded variety, the color of the seed is not affected, though the contained embryos are heterozygous, R(W). The seed accordingly is white, but plants raised from it bear red ears. And if another generation of plants is raised from such red seed, these prove to be of three sorts, as in the reciprocal cross, viz: 1RR, 2RW, and 1WW. The RR and RW plants bear red ears, the WW plants bear white ears, though all the plants alike were raised from red seed.

It follows that a red seed-coat may cover an embryo of any one of these sorts, RR, RW, or WW, but a white seed-coat may cover only two of these three sorts of embryos, viz: RW or WW. For the white seed must have received a maternal contribution of white, though the paternal contribution may have been either R or W.

If accordingly one selects seed by color alone from a mixed race of red and white, neither the red seed nor the white seed will breed true, either at the outset or after repeated selections. This fact, however, is not inconsistent with a strict Mendelian behavior of pericarp color in heredity. If in the supposed case selection is carried out on a considerable scale, we can predict with considerable accuracy what the proportion of red to white ears will be following each selection.

Reciprocal crosses between red seeded and white seeded varieties yield the same results so far as seed-color in the hybrid plants and in their offspring is concerned. The F_1 hybrid plants bear only red ears. Accordingly it is impossible in this generation to make any selection for seed-color. Some of the F_2 plants bear red ears, some white ears. Here then selection may begin. If one saves only white ears for seed in this and subsequent generations, the proportion of white to red ears in each successive crop should be approximately as shown in Table 1; if on the other hand one saves only red ears for seed, the proportions should be approximately as shown in Table 2.

A glance at these tables shows with what persistency a Mendelian character manifested as a maternal character only may be expected to crop out in the progeny of a mixed race, even when repeatedly excluded by selection. The dominant character, red,

TABLE 1

Expected results of selecting white ears only from a mixed race produced by a cross between a pure red and a pure white variety of maize

GENERATION	WHITE EARS		RED EARS	PER CENT WHITE	SELECTIONS MADE
F_1	0		all	0	0
F_2	1	:	3	25	0
F_3	1	:	1	50	1
F_4	3	:	1	75	2
F_5	7	:	1	87.5	3
F_6	15	:	1	93.7	4
F_7	31	:	1	96.8	5
F_8	63	:	1	98.4	6
F_9	127	:	1	99.2	7
F_{10}	255	:	1	99.6	8
F_{11}	511	:	1	99.8	9
F_{12}	1023	:	1	99.9	10

TABLE 2.

Expected results of selecting red ears only from a mixed race produced by a cross between a pure red and a pure white variety of maize

GENERATION	RED		WHITE	PER CENT RED	SELECTIONS MADE
F_1	all		0	100	0
F_2	3	:	1	75	0
F_3	5	:	1	83.3	1
F_4	7	:	1	87.5	2
F_5	9.1	:	1	90.4	3
F_6	12.3	:	1	92.5	4
F_7	15.5	:	1	94	5
F_8	19.0	:	1	95	6
F_9	23.4	:	1	95.9	7

has the lead at the outset, since all F_1, plants bear red ears, and this ascendency it holds through three successive selections, but beyond that point selection for the recessive character, white, takes the lead and this lead it increases at each successive selection. Nevertheless after ten selections have been made the white

series still produces one red ear in a thousand, and the red series produces a considerably larger proportion of white ears.[2]

Let us now compare with these series the results obtained by Miss McCracken in selecting for the conditions bivoltinism or univoltinism in a crossed race of silk-moths.

The original cross (1904) was made between a univoltin female and a male of bivoltin race. Ten of the F_1 female moths were tested, and six of these proved to be bivoltin, four univoltin in character. This looks like a Mendelian 1:1 ratio and suggests that one or the other parent in the original cross was heterozygous. But the results obtained by Toyama ('06) indicate that univoltinism is dominant over bivoltinism, and there is nothing in the results of Miss McCracken at variance with this idea. If so, the original female was a heterozygote, U (B), and when mated with a bivoltin male produced offspring half heterozygous, U (B), half pure bivoltin, BB. Matings of the F_1, offspring with pure bivoltins would have settled this point, but no such matings were made. We are left therefore with only such information as is afforded by five matings of the F_1 individuals *inter se* and by twenty-four matings of the F_1 males with univoltin females said to be of pure race.

These twenty-four females at any rate, proved to be all univoltin, but there is reason to think that not all of them were homozygous in that character. For of thirty tested females obtained from this cross, two proved to be bivoltin. Either, therefore, univoltinism is not always dominant over bivoltinism, or else one or more of these univoltin females of pure race was in reality heterozygous, U (B). There is nothing in this assumption at variance with the statement that bivoltinism did not occur in the race from which these twenty-four univoltin females came. For the results of Doncaster ('08) on Abraxas, of Miss Durham ('08) on canary-birds, and of De Vries ('08), on Oenothera show that in a wide variety of organisms one sex may be regularly heterozygous in gametic composition without resulting in the production of a single recessive individual, except in racial out-crosses.

[2] These tables show us what a serious task it would have been for our Puritan ancestors to eliminate from their harvests the occasional red ear which caused such joyous confusion at the New England husking-bees, even had their austere consciences demanded the undertaking.

If all the univoltin females employed in these twenty-four matings had been heterozygous univoltins, while their mates were half of them pure bivoltin, half heterozygous univoltin, then we should have expected the offspring to be as four bivoltin: five univoltin, or 1:1¼, instead of the observed 1: 14. It seems probable, therefore, that the twenty-four univoltin females were not all heterozygous, or else that the F_1 males mated with them were univoltin in character to a greater extent than their sisters, the tested F_1 females. The data given are insufficient for testing either hypothesis adequately. In either case we should expect the subsequent generation to contain a mixture of univoltin and of bivoltin females, as actually observed, but in what proportions they occurred would depend upon a number of contingencies. Concerning these we are largely without information, so that no Mendelian expectations of much value can be calculated. Nevertheless I have calculated one such set of expectations which is contained in Table 3. It is based on the following contingencies:

(1) That univoltinism is uniformly dominant over bivoltinism, and that, therefore, all bivoltin females transmit that condition only. But since the character of the male mate is in every case uncertain, it is assumed (2) that the males are in every generation of the same sorts as the females, and occur in the same proportions. (3) The actual mates of each group of females are assumed to have been such as one would obtain by random selection, that is they are univoltin and bivoltin in the same proportion as the population from which they are taken. If, for example, a group of individuals contains thirty univoltins and twenty bivoltins, and from this group five females are selected, it is assumed that 3 of them are univoltin and two bivoltin. (4) The F_1 offspring produced by the original cross are assumed to have been half pure bivoltins, half heterozygous univoltins. (5) The "pure" U females, mothers of series A, are assumed to have been heterozygous in one out of seven cases.

It will be seen from Table 3 that selection for B is, in nearly every case, attended by a reduction in the percentage of univoltins (increase in the percentage of bivoltins) as we should expect, though this reduction is less rapid than we should expect. Contrary to Miss McCracken's view, the bivoltins are not in excess,

but are deficient. On the other hand when selection is made for U, there is observed no increase in the percentage of univoltins. Such increase we should expect in a series of successive selections for U, but not of necessity as a result of a single selection. Thus in series E following a single selection of U females, we expect a smaller percentage of U females than in the parental series, A^1.

TABLE 3.

Percentages of univoltin females observed in the several series and their relations to the expected Mendelian percentages.

SERIES	NUMBER OF MOTHS TESTED	PER CENT UNIVOLTIN	PER CENT EXPECTED	RESULT OF
A′	30	93.3	94.6	
E	316	89.9	84.5*	1 selection of U
K	35	74.3	88.7	2 selections of U
H	209	85.2	51.4	1 selection of U, 1 of B
H′ (1909)	(?)	74	17.4	1 selection of U, 2 of B
B	6	40	50	
D	30	86 6	23.4	1 selection of B
G	23	69.6	12.1	2 selections of B
J	12	50	6.1	3 selections of B
J′ (1909)	(?)	33	3.1	4 selections of B
F	21	80.9	59.2	1 selection of B, 1 of U
L	11	72.7	70.2	1 selection of B, 2 of U
I	31	71	30.2	2 selections of B, 1 of U
I′(1909)	(?)	43	14.6	3 selections of B, 1 of U

*Miss McCracken's "Table of descent" is not in agreement with her text as regards the derivation of series E. I have assumed the correctness of the text, that series E is derived from the univolts (not the bivolts as shown) of series A′.

But we do not expect a second selection for U to be attended by a further decrease in the percentage of U females, as is observed in series K, and series L. This, as Miss McCracken observes, is a matter deserving explanation. It is unfortunate that we do not know from what particular broods the males were taken in each series. Without such knowledge a complete Mendelian analysis is impossible. If the parents of K and L happen to have been chosen from broods in which bivoltin individuals predominated, the high percentages of bivoltins in those series are fully explained.

On this point it is sufficient to quote a paragraph from Miss

McCracken's paper, p. 756. "In 1907, and again in 1908, precaution was taken to make a number of matings within each brood. It was found that all the tested females furnished by a few broods, particularly in 1908, were bivoltin-producing. Many of the broods furnished univoltin-producing females only and others furnished females part of whom were bivoltin-producing and part of whom were univoltin-producing. In each case these females were mated with males of similar ancestry." This is very clear evidence of Mendelian behavior of the characters univoltinism and bivoltinism. Had the author traced the descent through individual broods throughout her experiments instead of lumping them into series, I am confident she would never have characterized the inheritance as non-Mendelian. Even without this, had selection been made continuously for univoltinism within the mixed race, as was done for bivoltinism in one case, it can scarcely be doubted that the percentage of univoltins would have increased steadily, though probably less rapidly than bivoltinism in the reverse sort of selection. This at any rate is what we should expect if univoltinism is dominant. Compare Tables 1 and 2.

On the whole, notwithstanding the incompleteness of the data, we are, I believe, justified in concluding that univoltinism is a Mendelian dominant to bivoltinism. For when from a mixed race produced by crossing, selection is made for either condition, bivoltinism increases faster than univoltinism. The fact that bivoltin mothers may produce univoltin daughters when mated with males of unknown character is entirely in harmony with a Mendelian interpretation. It is unnecessary to assume a mysterious "pull of ancestry," a delayed "conjugation," or the "masking of an anlage" for a series of years followed by its reappearance, so long as a simpler explanation in line with established principles of inheritance fully accounts for the phenomena observed.

It may not be out of place to repeat that if one is to test fairly in a particular case the Mendelian or non-Mendelian character of inheritance, the line of descent must be known through individuals, not through masses of individuals. The futility of the mass-method of dealing with inheritance phenomena has been sufficiently illustrated in the results of the biometric school in England.

EFFECTS OF ALCOHOL ON THE LIFE CYCLE OF PARAMECIUM

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From the Biological Laboratory of Clark University

WITH ONE FIGURE

Calkins and Lieb (1) made some experiments with alcohol on paramecia and found that " . . . alcohol has no effect when taken in too weak doses, and too powerful an effect when taken in over strong doses." " . . . when a medium dose is given (for example 3 parts of $\frac{1}{1000}$ alcohol to 2 of hay, or 1 part of $\frac{1}{500}$ alcohol to 4 parts of hay) the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series." "There is no doubt that for a time at least, alcohol will prevent death during periods of depression." " . . . there is evidence that . . . the general vitality would decrease under the constant stimulus as it does under treatment with hay infusion alone, although much more slowly." "Notwithstanding the more rapid living, the general vitality does not seem to be affected badly by the alcohol."

Woodruff (2) found that:

1. "Minute doses of alcohol will decrease the rate of division at one period of the life cycle and increase it at another period of the life cycle."

2. "When alcohol increases the division rate, the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control, followed by fluctuations above and below the rate of the control."

Since the acute and chronic effects of alcohol on the Paramecium have not been clearly shown, these experiments were started with that end in view.

The single-celled animals are especially well adapted for the investigation of physiological activity. The ease with which they lend themselves to experimental methods and the simplicity of their structure make it possible to arrive at an accurate analysis of the effects of the stimulus in question.

This problem was suggested by Prof. C. F. Hodge under whose direction a series of similar experiments are now being carried on.

METHODS

Hay infusion was used at first as a culture-medium, but later it was found that alfalfa, on account of its uniformity, gave much better and more reliable results. A comparison of these culture-media is given in Table 1.

The infusion was made as follows: Fifteen grams of hay or alfalfa were boiled for one minute in 1500 cc. of tap water. This was prepared each day late in the afternoon and was used the next morning.

A "wild" paramecium was taken from a hay infusion in laboratory and placed in a watch glass in 8 cc. of fresh solution. At the end of twenty-four hours the hay solution was renewed. On the second day there were paramecia enough present to start the experiment.

At first, six cultures, each comprising four lines, were carried. Afterwards others were added. Using a pipet drawn out to a fine point, four individuals were transferred to four depression slides, each having a capacity of about five drops. Pure infusion was added and this started the control culture. In a similar manner cultures were started in the following percentages of alcohol, 1 per cent, $\frac{1}{10}$ per cent, $\frac{1}{25}$ per cent, $\frac{1}{50}$ per cent, $\frac{1}{100}$ per cent. A few days later cultures were started in 2 per cent, 3 per cent, 4 per cent, 5 per cent, and 6 per cent solutions.

In order to determine the resistance of paramecia to alcohol in greater quantities, the following experiment was performed. An individual was taken from the control and isolated in pure infusion. When it had made three divisions, a culture of four lines was started in pure solution for control. Another culture of four

lines was started in a ½ per cent alcohol infusion. This alcohol medium was increased by ½ of 1 per cent each day. The culture died when transferred into 9 per cent solution.

Separate pipets were kept for each culture and great care was taken to transfer as little infusion as possible along with each individual. A lens having a magnification of ten diameters was used entirely in transferring specimens with the pipet from one slide to another. This was easier than using a compound microscope.

The slides were kept in moist chambers to prevent evaporation of the infusion. These were stender dishes nine inches in diameter and three inches deep. In the bottom there was moist sand one inch deep on which rested four glass rods and on these rods were placed the depression slides. Then over all was placed the tight fitting cover. Cover glasses were not used on the depression slides.

Each day the rate of division was recorded for each of the cultures. When the count was made, an individual from each line was isolated on a clean depression slide in four drops of the culture medium. The four lines in each culture were averaged to get the daily rate of division, and this result was again averaged for eight day periods. In this way, fluctuations in division rate are eliminated and we have a more reliable and comprehensive result than would be obtained by carrying a single line.

The rate of division was taken as the index of the physiological condition of the organisms. All previous investigators in this field have considered this to be the most accurate indication which is available.

Believing that more reliable data could be had if the alcohol was mixed directly with the culture medium in the desired proportion, this plan was followed entirely. This method eliminates a possible source of error found in the "dropping" process which has been used extensively in similar experiments.

A COMPARISON OF HAY INFUSION AND ALFALFA INFUSION AS CULTURE-MEDIA

On January 6 the six cultures were started in ordinary hay infusion. From the first, the rate of division was low and it was plain

that something must be done to revive them. On January 19 the paramecia were transferred to an infusion made of alfalfa. They at once showed a marked increase in the rate of division. The division rate has since been uniformly high and devoid of depression periods, except one or two of very brief duration, due to fall of temperature in the laboratory.

TABLE 1

Table showing comparison of Hay Infusion and Alfalfa Infusion as Culture Media. The Alcohol was mixed directly with the media as indicated below. In column 1 are given the average generations of paramecia in hay infusion for thirteen days. Column 2 shows the average generations of the paramecia in alfalfa infusion during the succeeding thirteen days.

DAYS	CONTROL		1 PER CENT		$\frac{1}{10}$ PER CENT		$\frac{1}{25}$ PER CENT		$\frac{1}{50}$ PER CENT		$\frac{1}{100}$ PER CENT	
	(1)	(2)	1	2	1	2	1	2	1	2	1	2
1	1.25	1.25	2.25	1.25	1.	1.37	1.25	1.75	1.25	2.	1.	1.5
2	2.	2.50	2.75	2.75	2.25	2.49	1.25	3.50	2.	3.	1.75	3.
3	3.	4.25	3.25	4.25	2.50	3.61	2.50	4.50	3.	4.25	3.	4.5
4	4.25	6.25	4.	6.25	3.	5.36	3.75	6.37	4.	5.87	3.75	6.12
5	5.	8.12	4.5	8.25	3.5	6.86	4.75	8.62	5.	7.59	4.5	7.87
6	6.25	10.12	5.5	10.12	4.	9.11	6.62	10.68	7.	9.71	5.75	9.87
7	7.87	11.12	7.37	11.73	6.	10.86	7.87	11.68	8.25	10.83	7.25	10.12
8	8.62	12.87	8.12	13.23	6.5	12.36	9.12	13.68	9.25	12.45	8.25	12.12
9	9.62	14.62	8.87	14.73	7.5	13.86	9.87	16.36	10.	14.57	9.25	14.55
10	10.1	16.37	9.37	17.73	8.	16.36	9.87	19.36	10.2	17.57	10.1	17.21
11	10.8	17.80	10.1	19.48	8.25	17.36	9.87	20.36	12.	19.57	10.7	18.96
12	12.1	19.80	10.6	21.73	8.5	17.86	10.6	22.29	12.2	21.19	12.5	20.46
13	13.3	20.80	12.4	22.98	8.75	19.11	12.3	23.66	13.5	22.2	14.	21.46

GENERAL EFFECTS OF ALCOHOL ON THE DIVISION RATE

TABLE 2

Chronological table showing average generations of Paramecia in alcoholic alfalfa infusions. First experiment

DATE	CONTROL	1 PER CENT	$\frac{1}{10}$ PER CENT	$\frac{1}{25}$ PER CENT	$\frac{1}{50}$ PER CENT	$\frac{1}{100}$ PER CENT
Jan. 7	1.25	2.25	1.	1.25	1.25	1.
8	2.	2.75	2.25	1.25	2.	1.75
9	3.	3.25	2.5	2.5	3.	3.
10	4.25	4.	3.	3.75	4.	3.75
11	5.	4.5	3.5	4.75	5.	4.5
12	6.25	5.5	4.	6.62	7.	5.75
13	7.87	7.37	6.	7.87	8.25	7.25
14	8.62	8.12	6.5	8.98	9.25	7.25
15	9.62	8.87	7.5	9.87	10.	8.25
16	10.12	9.37	8.	9.87	10.25	9.25
17	10.87	10.12	8.25	9.87	12.	10.
18	10.87	10.62	8.5	9.87	12.25	10.75
19	12.12	12.49	8.75	10.62	13.5	12.5
20	13.37	13.74	10.12	12.37	14.5	14.
21	15.12	15.24	11.24	13.37	15.75	15.5
22	17.12	17.24	12.99	15.24	17.37	17.12
23	18.99	19.24	14.49	17.49	19.12	18.87
24	20.99	21.11	16.74	19.55	21.24	20.87
25	21.99	22.73	17.49	20.55	22.36	22.12
26	23.74	24.23	18.99	22.55	24.36	23.74
27	25.49	26.73	20.49	25.23	26.48	26.17
28	28.24	29.73	22.99	28.23	29.48	28.85
29	29.74	31.48	24.49	29.23	31.23	30.35
30	31.24	33.23	25.49	31.23	33.23	32.10
31	33.24	35.48	25.99	33.16	34.85	33.60
Feb. 1	34.74	36.73	26.24	34.53	36.6	35.35
2	35.74	37.48	27.49	35.53	37.6	36.35
3	37.24	39.48	28.49	37.53	39.1	37.85
4	38.74	40.98	29.74	39.28	40.1	39.35
5	40.49	42.23	30.99	40.28	41.35	40.1
6	42.24	43.73	32.74	42.53	42.6	43.16
7	44.49	45.73	34.24	44.78	45.1	45.33
8	45.24	46.73	35.24	45.53	45.85	46.58
9	46.99	48.6	37.24	47.28	47.51	48.58
10	48.99	50.6	38.99	49.15	49.38	50.
11	50.74	53.35	40.74	50.9	51.13	52.33
12	51.49	53.85	42.24	52.65	52.63	53.83

TABLE 2—*Continued*

DATE		CONTROL	1 PER CENT	$\frac{1}{10}$ PER CENT	$\frac{1}{25}$ PER CENT	$\frac{1}{50}$ PER CENT	$\frac{1}{100}$ PER CENT
Feb.	13	53.24	55.10	43.74	54.4	54.38	55.33
	14	55.24	57.16	45.74	56.4	56.25	57.7
	15	55.74	58.16	46.49	57.4	57.	58.2
	16	57.7	60.28	48.24	59.6	60.7	60.7
	17	59.74	62.	50.24	61.65	61.25	62.45
	18	60.5	63.	51.24	62.9	62.5	63.3
	19	63.11	65.	52.36	65.	64.5	64.8
	20	65.11	67.15	52.86	67.33	66.5	66.4
	21	67.11	69.2	53.8	69.2	68.8	68.92
	22	68.11	70.5	54.6	70.45	70.24	70.17
	23	70.11	72.5	56.6	72.45	71.89	72.17
	24	71.9	74.5	58.6	74.7	74.11	73.9
	25	73.9	76.5	60.73	77.45	76.36	76.17
	26	75.9	78.2	62.7	79.2	78.36	78.17
	27	77.9	80.2	64.7	81.2	80.4	80.4
	28	79.9	82.2	66.9	83.5	82.6	82.4
Mar.	1	81.1	83.2	67.9	84.23	83.6	83.4
	2	82.35	85.	69.4	84.7	85.1	84.9
	3	84.3	86.7	70.9	87.9	87.3	87.1
	4	85.3	87.7	71.9	89.2	88.6	88.1
	5	86.3	89.7	73.4	90.7	89.6	90.1
	6	88.1	90.2	74.6	92.4	91.1	91.6
	7	89.9	91.7	76.4	94.2	92.8	94.4
	8	91.2	93.2	77.6	95.4	93.8	95.7
	9	92.9	94.7	79.	97.2	95.6	97.4
	10	94.4	96.5	79.7	99.5	97.6	99.4
	11	96.2	98.	81.53	101.5	99.6	101.2
	12	98.2	100.	83.2	104.1	100.	102.9
	13	100.7	102.2	85.2	106.2	102.9	105.
	14	102.5	103.8	87.2	107.8	105.9	106.4
	15	103.5	104.6	88.2	109.6	105.9	107.8
	16	105.5	106.4	90.	111.1	107.4	109.2
	17	107.5	108.1	92.6	113.3	108.6	110.7
	18	109.4	110.1	94.1	115.1	109.6	111.9
	19	111.1	112.1	96.1	117.1	111.	113.9
	20	113.3	114.1	97.9	118.8	113.	115.9
	21	115.5	116.1	100.	121.3	115.6	118.2
	22	116.8	117.1	101.5	122.3	116.6	119.2
	23	118.5	119.1	103.2	124.3	118.6	121.3
	24	120.5	121.6	105.8	127.1	121.5	123.7
	25	121.8	123.6	107.8	129.1	123.2	125.7

TABLE 2—*Continued*

DATE		CONTROL	1 PER CENT	$\frac{1}{10}$ PER CENT	$\frac{1}{25}$ PER CENT	$\frac{1}{50}$ PER CENT	$\frac{1}{100}$ PER CENT
Mar.	26	123.	125.6	109.1	130.8	125.1	127.4
	27	124.9	127.3	110.6	132.8	127.	129.8
	28	126.8	129.3	111.6	134.9	129.1	131.9
	29	127.8	130.3	112.6	136.4	130.6	133.2
	30	129.	132.3	114.1	138.7	132.6	135.
	31	131.3	134.3	115.6	140.7	134.7	137.3
Apr.	1	133.	136.1	117.1	142.8	136.5	139.
	2	135.2	138.1	119.3	145.1	138.8	141.5
	3	137.	140.1	120.8	147.	140.6	143.3
	4	138.7	141.9	121.8	148.8	142.3	144.5
	5	140.	143.7	123.3	149.7	143.8	145.9
	6	140.7	144.7	124.6	150.9	145.1	146.9
	7	142.5	146.2	126.7	152.9	147.4	148.7
	8	145.	148.7	128.7	155.2	149.6	151.6
	9	146.5	150.8	130.6	157.3	151.6	153.7
	10	147.5	152.1	132.1	158.9	153.1	154.7
	11	148.3	152.6	132.3	159.4	153.6	155.2
	12	149.8	154.3	133.2	160.9	154.6	156.4
	13	151.5	155.8	134.6	162.8	156.4	158.4
	14	153.8	158.9	137.1	165.3	158.6	160.8
	15	156.1	160.7	138.1	167.5	160.4	162.
	16	158.9	162.7	138.8	169.2	162.1	163.5
	17	160.4	164.2	139.8	171.	163.6	165.2
	18	162.	166.7	141.1	172.8	164.9	167.4
	19	163.9	168.7	142.	174.6	166.7	168.8
	20	165.4	170.	143.8	176.8	168.	171.
	21	167.	172.	145.7	178.8	170.	172.8
	22	168.9	173.5	146.9	179.7	171.	174.3
	23	169.9	174.5	148.	182.	172.9	176.
	24	171.4	176.5	149.2	183.7	174.6	178.
	25	172.9	178.5	150.7	185.7	176.3	179.7
	26	174.	179.	151.9	186.9	177.3	180.7
	27	175.	180.5	152.6	188.1	178.3	181.7
	28	176.2	182.	153.8	189.1	179.4	183.
	29	178.	184.	155.4	190.7	180.6	184.5
	30	179.5	185.2	156.1	192.2	182.4	*
May	1	180.5	186.9	157.1	193.2	183.7	
	2	181.8	187.	157.8	195.2	185.3	
	3	183.3	188.6	158.4	196.1	186.	
	4	185.4	190.6	160.7	198.3	188.5	

*Discontinued.

TABLE 2—*Continued*

DATE		CONTROL	1 PER CENT	$\frac{1}{10}$ PER CENT	$\frac{1}{25}$ PER CENT	$\frac{1}{50}$ PER CENT
May	5	186.9	192.1	162.3	200.	190.
	6	188.4	193.6	164.1	201.3	191.6
	7	190.9	196.3	166.1	203.9	193.8
	8	192.4	198.	168.3	205.6	195.8
	9	194.4	200.	170.	207.1	197.3
	10	195.4	201.7	171.	208.6	198.3
	11	197.4	203.7	173.	210.3	200.
	12	199.6	205.7	175.	212.3	202.
	13	201.9	208.	176.7	215.	205.
	14	204.4	210.	178.	217.3	207.3
	15	206.4	211.	179.2	219.1	209.
	16	208.4	213.	180.9	220.8	211.
	17	210.4	215.	182.	222.	212.6
	18	212.4	217.	184.	224.	214.6
	19	214.1	218.3	185.3	225.	216.6
	20	215.6	219.7	186.	226.2	217.6
	21	216.6	220.5	187.5	227.2	218.6
	22	217.6	221.5	189.5	228.2	219.6
	23	218.6	222.5	189.5	229.2	221.6
	24	220.6	224.5	191.5	230.2	222.9
	25	222.6	226.8	193.3	231.7	224.4
	26	224.6	228.3	195.1	233.3	227.
	27	226.1	230.6	196.4	235.6	*
	28	228.1	232.6	197.7	237.6	
	29	230.1	233.9	199.	238.6	
	30	232.1	235.6	201.	240.6	
	31	234.1	238.1	203.5	242.6	
June	1	236.6	240.1	206.	243.6	
	2	238.6	242.1	207.5	244.6	
	3	240.6	244.1	209.6	246.9	
	4	243.	245.6	211.6	248.	

* Discontinued.

TABLE 3

Chronological table showing average generations of Paramecia in alcoholic alfalfa infusions. Second experiment.

DATE		CONTROL	2 PER CENT	3 PER CENT	4 PER CENT	5 PER CENT	6 PER CENT
Jan.	26	1.7	2.2	1.	1.5	1.	1.
	27	3.5	4.8	3.	2.5	1.5	1.7
	28	6.2	7.8	5.	3.7	3.3	1.7
	29	7.7	9.2	6.2	4.7	5.3	2.5
	30	9.2	10.9	8.7	5.7	7.3	4.
	31	11.2	12.7	9.9	6.7	8.3	5.2
Feb.	1	12.2	14.4	10.1	7.9	9.5	5.7
	2	13.2	15.4	10.6	9.1	10.7	6.5
	3	14.7	17.3	10.8	10.2	13.	6.8
	4	16.2	19.3	11.8	11.4	14.	7.8
	5	17.9	20.6	13.3	12.6	15.5	8.3
	6	19.6	22.1	13.8	13.4	17.	8.3
	7	21.8	24.2	15.8	14.7	18.	8.6
	8	22.6	25.5	17.1	15.9	19.3	8.6
	9	24.4	26.9	18.6	17.5	20.	Died
	10	26.4	28.	20.1	19.	21.	
	11	28.1	29.7	21.3	20.3	21.8	
	12	29.6	31.5	23.1	21.3	22.8	
	13	30.6	32 8	23.8	21.8	23.3	
	14	32.6	34.8	25.6	24.3	23.6	
	15	35.8	35.8	27.3	25.5	23.9	
	16	35.8	37.9	28.3	26.5	24.4	
	17	37.8	39.9	30.3	28.7	24.9	
	18	39.5	40.7	32.6	30.7	25.4	
	19	42.1	42.9	34.8	33.2	Died	
	20	44.1	45.3	35.8	34.		
	21	46.1	47.5	37.3	36.		
	22	47.1	48.8	39.3	37.6		
	23	49.1	50.8	40.6	39.4		
	24	50.9	52.8	42.6	40.9		
	25	52.9	55.	44.8	42.2		
	26	55.1	57.	46.4	44.1		
	27	57.1	58.5	47.1	45.1		
	28	59.1	60.5	48.1	45.6		
Mar.	1	60.2	61.	49.6	47.6		
	2	61.5	61.8	50.6	48.6		
	3	63.5	64.3	52.1	50.6		
	4	64.5	65.7	53.6	51.8		
	5	65.5	67.4	55. 2	53.3		
	6	67.2	69.2	56.4	54.		
	7	69.	70.5	57.6	55.		
	8	70.2	71.5	59.3	55.		

TABLE 3—*Continued*

DATE		CONTROL	2 PER CENT	3 PER CENT	4 PER CENT		
Mar.	9	72.	73.3	61.3	55.3		
	10	73.5	75.	62.8	55.8		
	11	75.2	76.3	65.3	56.3		
	12	77.2	77.5	66.3	56.6		
	13	79.7	79.	67.3	56.6		
	14	81.5	0.5	68.6	56.9		
	15	82.5	81.9	70.	57.7		
	16	84.5	83.4	72.	Died		
	17	86.5	85.	73.			
	18	88.3	86.5	75.			
	19	90.	88.5	77.			
	20	92.2	90.5	78.			
	21	94.4	92.5	80.			
	22	95.7	93.9	82.2			
	23	97.4	97.	83.4			
	24	99.4	98.5	84.2			
	25	100.6	99.7	86.			
	26	101.9	101.	87.			
	27	103.8	102.	87.8			
	28	105.6	104.2	89.			
	29	106.6	105.4	90.5			
	30	107.8	107.3	91.8			
	31	110.	109.4	93.3			
Apr.	1	111.7	111.3	94.7			
	2	113.9	113.4	96.2			
	3	115.7	115.3	97.9			
	4	117.5	117.3	98.9			
	5	118.9	118.9	101.2			
	6	119.7	119.7	103.2			
	7	121.5	121.2	105.4			
	8	124.	123.2	106.2			
	9	125.5	125.5	107.2			
	10	126.5	126.5	108.			
	11	127.3	128.7	109.3			
	12	128.8	128.7	110.8			
	13	130.6	130.8	112.8			
	14	132.8	133.	114.8			
	15	135.2	134.8	116.3			
	16	136.9	136.8	117.8			
	17	138.4	138.5	119.3			
	18	140.1	140.6	121.			
	19	141.8	141.9	123.			
	20	143.3	143.6	123.7			
	21	145.8	146.1	124.0			

TABLE 3—*Continued*

DATE		CONTROL	2 PER CENT	3 PER CENT			
Apr.	22	146.8	147.3	126.6			
	23	147.8	148.8	128.6			
	24	149.6	150.6	129.6			
	25	150.8	152.6	130.3			
	26	151.8	153.6	131.6			
	27	152.8	154.8	132.8			
	28	154.1	156.1	133.8			
	29	155.8	157.3	135.8			
	30	157.4	158.3	137.1			
May	1	158.4	160.2	138.4			
	2	159.8	161.4	140.7			
	3	161.1	162.9	142.2			
	4	163.2	165.4	143.7			
	5	164.7	167.1	146.			
	6	166.3	168.6	147.5			
	7	168.8	170.9	149.5			
	8	170.6	172.1	150.7			
	9	172.6	174.3	152.7			
	10	173.6	175.8	154.7			
	11	175.6	177.8	158.			
	12	177.8	179.8	160.			
	13	180.	182.3	162.			
	14	182.5	184.6	164.			
	15	184.5	186.1	166.			
	16	186.5	187.9	168.			
	17	188.5	189.7	170.			
	18	190.5	192.2	172.5			
	19	192.3	194.	173.5			
	20	193.8	195.5	175.			
	21	194 8	196.5	176.			
	22	195.8	197.5	177.			
	23	196.8	198.5	178.			
	24	198.8	200.5	180.			
	25	200.8	202.5	182.			
	26	202.8	204.3	183.5			
	27	204.3	205.8	85.			
	28	206.3	207.8	186.5			
	29	207.3	209.	187.5			
	30	209.3	210.	189.			
	31	211.3	213.	191.			
June	1	213.3	215.5	193.			
	2	215.8	217.5	194.			
	3	218.	219.5	195.5			
	4	220.	221.5	196.5			

EXPERIMENTS WITH ALCOHOL

The first series of experiments was started January 6 and carried on until June 4. The percentage of alcohol given, varied from 1 per cent to $\frac{1}{100}$ per cent.

The different cultures, with one exception, showed remarkable uniformity in division rate. The exception was the culture in $\frac{1}{10}$ per cent infusion. From the very beginning and uniformly throughout the experiment these individuals showed a slower rate of division. But since there were three cultures in weaker alcohol solutions and all of them equaled the control in division rate, we conclude that this exception was caused by sources other than the alcohol.

When the experiment was discontinued the control had reached 243 generations, the 1 per cent culture 245, the $\frac{1}{10}$ per cent culture 211, and the $\frac{1}{25}$ per cent culture 248. The $\frac{1}{50}$ per cent culture was discontinued in the 227th generation at which time the control was in the 224th. The 1–100 per cent culture was discontinued in the 184th generation while the control was in the 178th.

The second series of experiments was started January 26. The percentage of alcohol varied from 2 per cent to 6 per cent. At the end of the 15th day, the 6 per cent culture was dead. During this time it had reached only the 8th generation, while the controlwas in the 22nd. The 5 per cent culture lived 25 days and reached the 25th generation while the control was in the 39th. The 4 per cent culture died during the 50th day in the 57th generation at which time the control was in the 82nd generation.

When the 6 per cent culture died it was 14 generations behind the control, the 5 per cent culture was also 14, and the 4 per cent culture was 25.

The experiments were discontinued on June 4. The 3 per cent culture had reached 196 generations and was 24 generations behind the control. It might be noted here that this culture seems to mark the beginning of the effects of alcohol. The 2 per cent culture was in the 221st generation, and showed no effects whatever from the stimulus. The control was in the 220th generation.

Depression periods were never in evidence. When the tempera-

ture of the laboratory was allowed to run low on holidays and Sundays there was a marked lowering of the division rate *which affected all the cultures alike*. This temporary check always disappeared when the temperature came back to the normal state.

CONCLUSIONS

Our experiments show that:

1. There is no evidence that alcohol acts as a periodic or continued stimulus.
2. There is no evidence that the general vitality would decrease under the constant stimulus of minute doses.
3. Alcohol in minute doses, 2 per cent or less, has no effect whatever.
4. When a medium dose is given, for example 3 per cent, the general vitality is weakened.
5. If alcohol is given in greater strength than 3 per cent the rate of division is lowered and the organisms finally die.

LITERATURE

(1) Gary N. Calkins and C. C. Lieb '02—Studies on the Life-History of Protozoa.
2. The Effects of Stimuli on the Life-Cycle of Paramecium caudatum, Archiv für Protistenkunde.

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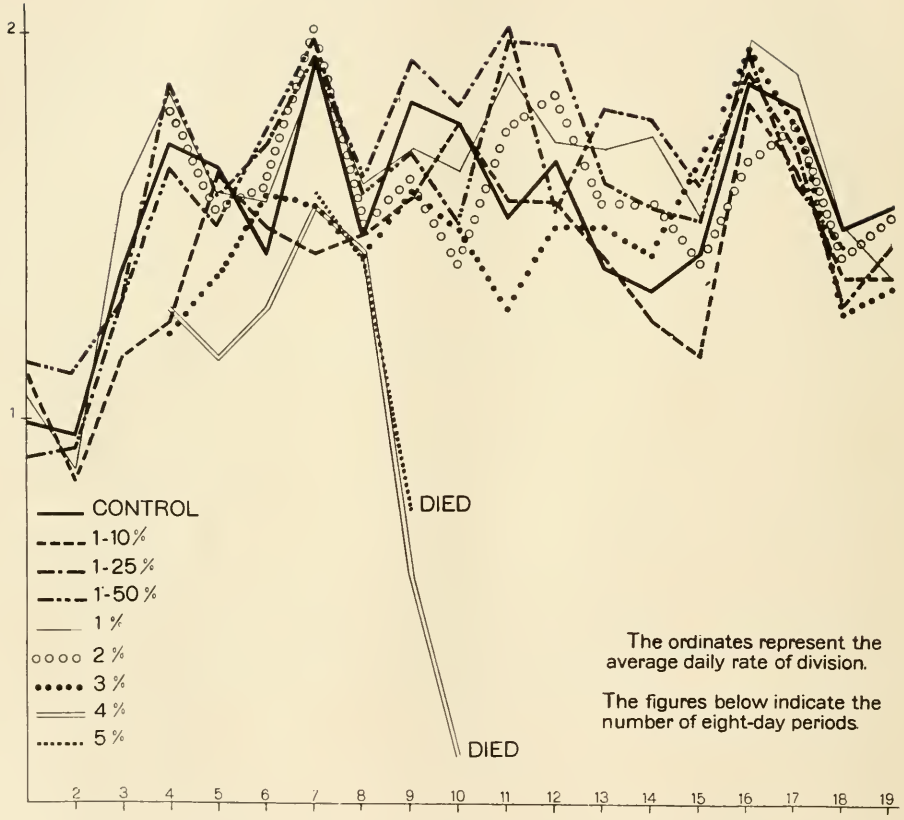

Chart showing division rate of cultures averaged for eight day periods.

THE CHROMOSOMES IN THE GERM-CELLS OF CULEX

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With Fifty-two Figures

In the summer of 1905, Miss Boring and I collected material for the study of the spermatogenesis of the mosquito, but the germ-glands proved not to be sufficiently well fixed. In 1907 I spent several days studying aceto-carmine preparations from the larvæ and pupæ of some California mosquitoes. Naturally I expected to find one or more heterochromosomes, but nothing of the kind could be detected either in the growth stages of the spermatocytes or in the maturation divisions. The number of chromosomes was small, only three in one species and four in the other, but it was not an easy matter to determine whether or not the pairs of univalents were exactly equal.

In October of this year (1909) I accidentally discovered an abundance of larvæ and pupæ of Culex (sp.[1] not determined, probably C. pungens), in a small pond where I was able to collect the material up to November 22. This time I determined the location of the testes and ovaries, in the third segment from the end of the tail—removed the anterior segments, and secured good fixation in Flemming's fluid and fairly good in Gilson's mercuro-nitric. The larger part of the material was dissected and the germ-cells studied in aceto-carmine preparations. With careful sealing it has been found that these slides can be kept in usable condition

[1] April 7, 1910. It is now quite certain that pupæ of two species were used in this work. All of the material that can be obtained from the same pool this season will be examined with a view to determining the conditions in the germ cells of each species breeding there. A species of Anopheles with 6 chromosomes in the spermatocytes has already been found.

for several weeks, if the material has not been too deeply stained in the beginning. The fixed material was stained either with iron-hæmatoxylin or with thionin, both giving good results especially with the Flemming fixation. Figures of cells and chromosomes drawn from sections are only about two-thirds as large as those taken from aceto-carmine preparations. The difference is mainly due to shrinkage in fixing fluids and alcohols, though the acetic acid probably swells the structures slightly. The majority of the figures were taken from aceto-carmine preparations and those taken from sections will be designated as such.

A few oögonia were found in mitosis in various young ovaries. In all cases the chromosomes were paired in prophases and metaphases before metakinesis (Figs. 1 and 2), as previously described by the author in several species of Muscidæ ('08). Two longer pairs are present with one pair considerably shorter. The homologous chromosomes composing the pairs are apparently equal in length. As in the Muscidæ, each of the six chromosomes divides longitudinally, and pairing of the daughter chromosomes probably occurs in the telophase, for very early prophases show the chromosomes paired and twisted together forming three spireme threads which gradually shorten and separate for mitosis. Fig. 3 shows the chromosomes of an oöcyte in an early growth stage with the paired chromosomes still distinct, and Fig. 4 the nucleus of a somewhat later stage showing three separate spireme threads of different lengths. Whether these separate spiremes later unite to form a single thread I have been unable to determine, but that parasynapsis occurs immediately after the last oögonial mitosis is certain, and it is equally certain that the chromosomes are similarly paired in earlier generations of the oögonia.

Fig. 5 is an outline camera drawing of a testis stained in aceto-carmine and considerably flattened under the cover-glass. In the first cyst at the tip (*a*) were resting spermatogonia, in the second (*b*) anaphases and telophases of spermatogonial mitoses. Then followed cysts (*c*, *d*, *e*) containing synizesis stages, and growth stages of the first spermatocytes, one cyst (*f*) in a stage immediately following the first maturation division, several cysts of spermatids (*g*, *h*, *i*, *j*) and masses of spermatozoa (*k*) pressed out through

the broken wall of the testis. The two testes are situated one on each side of the digestive tract in the third segment from the end of the tail of the pupa. Maturation occurs mainly, if not wholly, during the pupa stage.

Fig. 6 is a good specimen of an early prophase of spermatogonial mitosis, showing three long granular chromatin threads, one of which already shows its double character. Fig. 7 is a section of a nucleus showing the twisted chromosomes of a later prophase stage. Fig. 8 is a spermatogonium in metaphase, showing the pairs separated and apparently all equal; Fig. 9 the chromosomes from a similar stage, in outline, so as to show the full length of each chromosome; and Fig. 10 a late prophase from a Flemming-iron-hæmatoxylin section. In no one of these figures would one suspect that one pair of chromosomes might be unequal, but in the testes of two individuals I found seven plates of a different character, one of which is shown in Fig. 11, with the shorter pair of chromosomes apparently composite, each consisting of a longer and a shorter portion, the longer components equal, and the shorter unequal and suggesting a case of unequal heterochromosomes such as occur in the Muscidæ ('08). Had I not found these cases, six in one testis and one in another, I should have said that there was no evidence in the mosquitoes of any such heterochromosomes as occur in so many other insects, and are clearly present in the nearly related Muscidæ.

A very distinct synizesis stage occurs in which the granular and beaded chromatin threads are wound about a large nucleolus, which in Flemming material stained with thionin, is yellowish in early stages and gradually acquires a staining quality nearly equal to that of the chromosomes. Figs. 12, 13 and 14 were taken from the same section to show an early synizesis stage with a pale plasmosome, a later stage with blue-staining plasmosome, and a pale spireme stage with the plasmosome stained a deep blue (Fig. 14). This series suggests an extrusion of chromatin substance from the spireme during the synizesis stage and an absorbtion of the extruded material by the plasmosome. In Culex it is quite certain that parasynapsis occurs in each cell generation

of the germ cells in the telophase. Other cases[2] where synapsis is known to occur either before or after synizesis have indicated that the two phenomena have no necessary connection. In a recent paper Miss King ('08) describes a separation of chromatin substances and rejection of masses of deeply staining material from the spireme during synizesis in the oöcytes of Bufo lentinginosus. Something similar was observed by Miss Boring ('07) in connection with the synizesis stage of the spermatocytes of three species of Ceresa (Pl. III, Figs. 62–67, 82 and 93). In the former case the rejected chromatin substances were observed in the form of nucleoli and also as a deposit on the nuclear membrane; in Ceresa they formed a dense plate at the base of the bouquet of short chromatin loops in the synizesis stage, and later became divided up into several dense masses distributed over the inside of the nuclear membrane and gradually disappearing in the later growth stages and prophases of the first maturation division. Buchner also describes a "Chromidial-austritt" during the synizesis stages of the oöcytes of Gryllus campestris where granules of material staining like chromatin are extruded from the nucleus in the region where the ends of the chromatin loops touch the nuclear membrane ('09, Pl. 21, Figs. 119–121). The change in staining quality of the plasmosome in Culex may therefore be regarded as further evidence that the synizesis stage of both oöcytes and spermatocytes is probably a period during which some modification of the chromatin occurs preliminary to maturation. Whether the rejected material visible in some cases, is waste material or substances which have some function connected with the growth stages of the germ cells, we can only surmise.

All through the synizesis and growth stages of the spermatocytes of Culex, there is absolutely no sign of any condensed heterochromosomes, only a plasmosome and a spireme, or perhaps three separate spireme threads. When the spireme begins to shorten and thicken one can occasionally be sure that it is not

[2] As examples where synapsis occurs before synizesis, I might cite from my own work, Photinus pennsylvanicus and Limoneus griseus ('09, Pl. I, Fig. 5–8; Pl. II, Figs. 31–38), while in many other species among the Coleoptera synapsis occurs at the close of the synizesis stage as in Chelymorpha argus ('06, Pl. IX, Figs. 37–43) and Photinus consanguineus ('09, Pl. I, Figs. 23 and 24).

continuous. In Fig. 15 one double end was seen above the plasmosome, and a segment with both ends free at a lower focus. From this stage on, the three chromatin threads shorten, thicken, and each separates into its two parallel components. Fig. 16 is an early prophase showing the three long twisted pairs, Fig. 17 a later stage with shorter, thicker twists, and Fig. 18 a slightly later stage from a section, showing a very characteristic appearance of the three pairs about the time that the spindle is formed. Fig. 19 is also from a section, and shows the plasmosome still present but pale again. In both testes from one individual, examined in aceto-carmine preparations, there was one cyst of prophase stages, two of which are shown in Figs. 20 and 21, where one pair of chromosomes was condensed while the others were still pale and granular. The material was collected on November 22, after freezing weather, and a very unusual number of cells were in the prophase and metaphase of the first maturation mitosis. The chromosomes and cells were both smaller than usual, and I thought that maturation must have been hastened by high temperature following cold, and that the stages shown in Figs. 20 and 21 were abnormal. Later, however, I found a trace of the same phenomenon in perfectly normal material well fixed with Flemming and stained with thionin; i. e., one pair of chromosomes becoming condensed in advance of the other two. The condensed pair in Fig. 21 resembles closely the shorter pair in Fig. 18 and other similar stages; and, together with the inequality observed in seven spermatogonial equatorial plates (Fig. 11), indicates that the smaller pair of chromosomes in Culex may have some of the characteristics of the heterochromosomes of other insects.

Fig. 22 is a typical first spermatocyte in metaphase or metakinesis, the spindle being formed within the elongated nucleus. In Fig. 23 the same chromosomes are shown separately. The middle one (*b*) is the shorter pair of the spermatogonia and prophase stages and the ring is the figure 8 of Fig. 18, *b*. In rare cases all three pairs may come into the spindle in the form of rings, but usually only one pair takes this form. Fig. 24 is a very frequent prophase appearance, showing one ring with over-

lapping ends, and the other pairs, one of them simply crossed, the other changing from the parasynapsis arrangement of the earlier prophase to the telosynapsis method of union of the chromosomes in metaphase. Fig. 25, *a* and *b*, shows a different method of union of the ends. They most often overlap, but may unite and split giving the cross-form so familiar in insect spermatogenesis. Fig. 26 shows an unusually well marked cross; it is the same ring-shaped chromosome with the outer ends separated, the other pairs being already in metakinesis. Fig. 27 is a later stage showing the ring chromosome about to separate later than the other pairs. Figs. 28 and 29 show again both methods of union of the chromosomes in telosynapsis. Fig. 30 is an extreme case of overlapping. Figs. 31 and 32 show two other groups, in each case the group of three being from the same spindle. Fig. 33 is a rare case in which all three pairs appeared as rings in the spindle. Figs. 34 and 35 are prophase and metaphase stages from sections. The anaphase in Fig. 36 shows that the overlapping ends of a pair of chromosomes may remain attached side by side until quite a late anaphase instead of pulling out into the end to end position seen in Figs. 23 and 31. It is interesting to find in Culex a clear case of parasynapsis in oögonia, oöcytes, spermatogonia and spermatocyte prophases, and then to see these same chromosome pairs appearing in the first maturation metakinesis as though united end to end (telosynapsis), and not only this, but to be able to trace all the changes from parasynapsis to telosynapsis in some of the preparations. In the Muscidæ the chromosomes pair side to side (parasynapsis), and separate in the first maturation mitosis in a manner closely resembling many cases of longitudinal division, going to the poles in the form of V's while in Culex the pairs become more or less perfectly united end to end (telosynapsis) and then separate as V's. In many cases one can only infer from the position of a pair of chromosomes in the spindle what the method of synapsis has been. In Culex, if one saw only the metaphases and anaphases one would certainly say that it was an undoubted case of telosynapsis, but the fact is that we have here a case of intimate and prolonged parasynapsis somewhat similar to that observed by Strasburger and his school

in both somatic and germ cells of various plants, the clearest cases being described by Overton ('09) in Thalictrum purpurascens and Calycanthus floridus.

In a number of spindles, most of them from one testis, the left hand chromosome pair (*a*) of Fig. 23 was found irregularly fragmented (Figs. 37, 38, 39), and even unequally divided as in Figs. 40 and 41. These cases of fragmentation were very rare and were found among a larger number of cells containing normal groups of chromosomes, but constrictions such as appear in Fig. 23, *a*, were frequent. Fig. 42 shows a still deeper constriction in the same chromosome. These cases of fragmentation and constriction suggest that this particular chromosome pair may be composite, and I should therefore not be surprised to find other species of Culex with a larger number of pairs of smaller chromosomes, or even to find more than the expected number in somatic cells of this species.

In the telophase of the first maturation division the chromosome fuse and then large vacuoles appear as in Figs. 43 and 44. Later one finds a distinct nuclear membrane and chromosomes lying on this membrane as in Fig. 45, where cell *a* is drawn to show an optical section through the nucleus, and cell *b* a tangential section. In the prophase of the second division (Fig. 46) the chromosomes are already divided longitudinally, and they always come into the spindle divided and much tangled (Fig. 47). As in the first division the spindle forms in the elongated nucleus. In the anaphase the V-shaped chromosomes move out of the tangled metaphase and form regular polar groups (Figs. 48 and 49). A telophase is shown in Fig. 50.

As a final effort to decide whether the smaller pair of chromosomes is equal or unequal, I went through my sections again and made camera drawings of the plainest cases of prophase grouping and of the various metaphase forms (Figs. 51 and 52). The components of the pair in prophase are always more or less twisted and foreshortened, so that a slight difference like that indicated in Fig. 11 might be difficult to detect. In Fig. 51, *a* to *d* are from prophases of the first spermatocyte, *e* from a late spermatogonial prophase. Similar cases may be seen in the metaphases

of Figs. 8 and 9. In all of these cases I should suppose that the pairs were equal if the question had not been raised by the seven spermatogonial plates represented by Fig. 11, and also by the occurrence of an unequal pair of heterochromosomes in each of the nine species of Muscidæ previously described ('08). In Fig. 52, *a* to *f*, the same pair of chromosomes is shown in metaphase and metakinesis. It is possible in each case that one chromosome is slightly larger than its mate, but the difference is certainly not conspicuous, and, if present, is obscured by the fact that the outer, free ends of the elements usually turn in different directions.

DISCUSSION

Heterochromosomes

If we define a heterochromosome as one that remains condensed through the growth stages of oöcytes or spermatocytes, then we must say that such are not present in Culex. We have seen, however, that one pair of chromosomes may be condensed in advance of the other two pairs, in an early prophase of the first maturation mitosis, and in the spermatogonia of two individuals we have found evidence that an unequal pair of small chromosomes is combined with a larger equal pair, which, we would suggest, may control the behavior of the smaller unequal heterochromosome pair, preventing it from remaining condensed during the growth stage. On the other hand the tendency of the heterochromosomes to remain condensed may account for this pair of chromosomes sometimes appearing in condensed form earlier than the other two pairs in the prophase of the first maturation division. It is certain that in most cases the heterochromosomes, if present, are so intimately fused with another pair of chromosomes that it is rarely possible to detect their presence, the slight difference in length of a pair of long, twisted chromosomes being difficult to determine.

The case of Culex is an interesting one in connection with that of several species of Lepidoptera (Stevens '06, Dederer '07), Nezara (Wilson '05) and Forficula[3] (Zweiger '06) and Anisolabis

[3] In the case of Forficula auricularia, the author finds an unequal pair of heterochromosomes entirely distinct from the lagging pair described by Zweiger as present in some individuals and absent in others.

(Randolph '08) in which an equal pair of chromosomes has been described as resembling the odd heterochromosome and the unequal heterochromosome-pair of other insects, in that it remains condensed during the growth stage of the spermatocytes. The conditions in Culex would indicate that in other cases where no heterochromosomes have been found, they may nevertheless be present, combined with other chromosomes in such a way that a slight inequality in size may easily escape detection, and that their characteristic behavior during the growth stage of the spermatocytes may be changed by the influence of the pair with which they are combined. As to the relation of the heterochromosomes to sex determination further discussion seems to be of little value until we get more evidence in connection with experimental breeding.

Synizesis

The synizesis stages of Culex apparently have no relation whatever to the phenomena of synapsis, but are interesting in that they afford further evidence that synizesis is a period of reconstruction in which certain elements present in the spermatogonial chromosomes are either rejected as waste material or are isolated in order that they may perform some function in connection with the growth stages of the germ-cells.

Synapsis

Perhaps the most interesting point in the history of the germ-cells of Culex is the fact that, as in the Muscidæ, pairing, or synapsis, occurs in connection with each spermatogonial and oögonial mitosis as well as in anticipation of maturation. I have not been able to study somatic mitoses in Culex, but in the Muscidæ a similar pairing was found in follicle cells of the ovaries, and it may therefore be true that pairing of homologous chromosomes occurs in connection with each mitosis throughout the life history of these insects, as Overton thinks probable in the case of several of the higher flowering plants whose cytology he has studied. Both in the Muscidæ and in Culex, all the evidence indicates

that parasynapsis of homologous chromosomes occurs in the telophase of each mitosis of the germ-cells, and that this intimate relation of the paternal and maternal members of the pairs persists from one mitosis to the next, when in the oögonia and spermatogonia, each chromosome divides longitudinally, but in maturation the two members of a pair separate and go to different cells. It is of especial interest to see in Culex a perfectly clear case of parasynapsis change in some cases to an equally clear case of telosynapsis before metakinesis, while intermediate ring-stages and cases of overlapping ends also occur.

SUMMARY

1 The number of chromosomes in Culex sp. is six in oögonia and spermatogonia and three in the spermatocytes.

2 There is some evidence that a small unequal pair of heterochromosomes is combined with a larger equal pair of chromosomes.

3 During the synizesis stage, the staining quality of the plasmosome changes in such a way as to indicate that it has received chromatin material from the chromatin threads wound about it.

4 Parasynapsis occurs in each cell-generation of the germ-cells, the homologous maternal and paternal chromosomes being paired in telophase and remaining so until the metaphase of the next mitosis.

5 Parasynapsis of homologous chromosomes often changes to telosynapsis in the metaphase of the first spermatocyte.

Bryn Mawr College
December 20, 1909[1]

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Description of Figures

The figures were all drawn with camera lucida. Fig. 5 was drawn with Zeiss 16 mm. compens. 4; all others figures with Zeiss 1.5 mm, compens. oc. 12. The plates were reduced one-fourth.

Fig. 1 Oögonium showing 3 pairs of chromosomes in equatorial plate. Ac-c-prep.

Fig. 2 Three pairs of chromosomes from another oögonium. Ac-c.

Fig. 3 Chromosomes and plasmosome (*p*) from a young oöcyte. Ac–c.

Fig. 4 Nucleus of an older oöcyte showing 3 separate spireme threads. Ac-c.

Fig. 5 Outline camera drawing of a testis mounted in aceto-carmine and flattened by pressure on the cover-glass. *a* = cyst of resting spermatogonia; *b* = spermatogonia in anaphases and telophases; *c*, *d*, *e* = synizesis stages; *f* = second spermatocytes in stage seen in Fig. 44; *g*, *h*, *i*, *j* = spermatids; *k* = masses of spermatozoa. Ac-c.

Fig. 6 Spermatogonium from tip of testis, early prophase showing one chromatin thread separating into its component chromosomes Ac-c.

Fig. 7 Section of a nucleus of a spermatogonium showing twisted pairs of chromosomes in prophase of mitosis.

Fig. 8 Spermatogonium showing three pairs of chromosomes in metaphase Ac-c.

Fig. 9 Chromosomes from another spermatogonium in outline. Ac-c.

Fig. 10 Section of a spermatogonium showing all three pairs of chromosomes in late prophase.

Fig. 11 Chromosomes from one of 7 equatorial plates found in spermatogonial cysts in two individuals. The smaller pair of chromosomes appears to be composed of an equal and an unequal pair combined. Ac-c.

Fig. 12. Early synizeses stage from material fixed in Flemming and stained in thionin Plasmosome (*p*) pale yellowish. Sec.

Fig. 13 Later synizesis stage from same section, plasmosome (*p*) blue

Fig. 14 Spireme growth stage from same section, plasmosome deep blue.

Fig. 15 Late growth stage of first spermatocyte, focusing from the surface of the nucleus down to the plasmosome (*p*), and showing one end of a spireme double. Ac-c.

Fig. 16 Prophase of a first spermatocyte mitosis showing the three twisted pairs of chromosomes. Ac–c.

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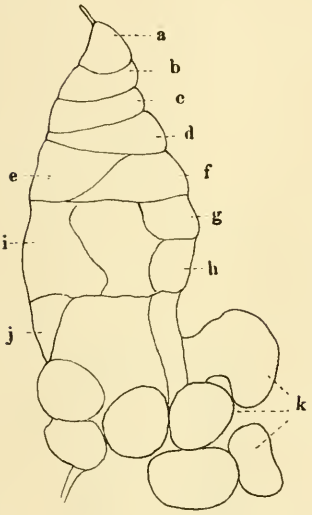

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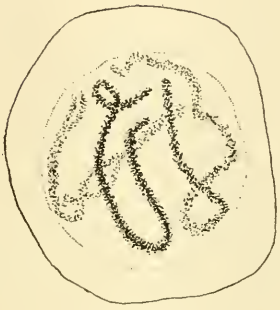

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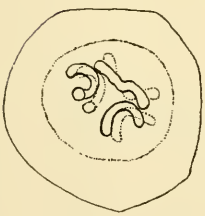

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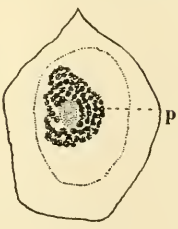

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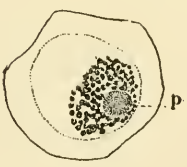

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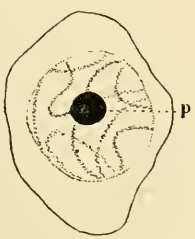

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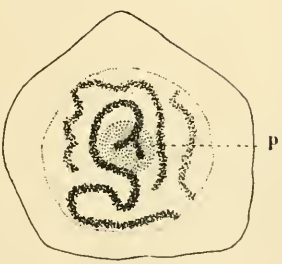

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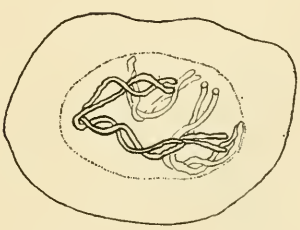

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Description of Figures

Fig. 17 Later prophase. Ac-c.

Fig. 18 Still later prophase from a section; showing the shorter pair (*a*), a figure 8 (*b*) which later appears as a ring, and a second long pair (*c*).

Fig. 19 Section of a prophase showing the plasmosome (p) still present.

Figs. 20 and 21 Exceptional prophases in which one pair, apparently the shorter one, was completely condensed earlier than the other two pairs. Ac-c.

Fig. 22 Typical first spermatocyte metaphase. Ac-c.

Fig. 23 Chromosome bivalents from the same cell drawn separately. Ac-c.

Fig. 24 Chromosomes from a slightly earlier stage, a spindle prophase. Ac-c.

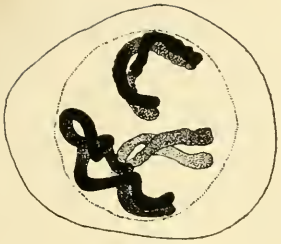
Fig. 25 *a* and *b* The ring chromosome. Ac-c.

Fig. 26 Three chromosome pairs from one spindle showing the ring-chromosome forming a cross where the ends are united. Ac-c.

Figs. 27, 28, 29 Other groups of chromosomes, each group from one spindle. Ac-c.

Fig. 30 Pair of chromosomes showing extreme case of overlapping, from a metaphase. Ac-c.

Fig. 31 Another metaphase group. Ac-c.



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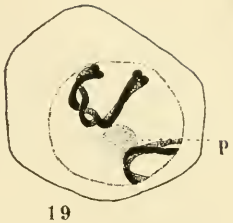

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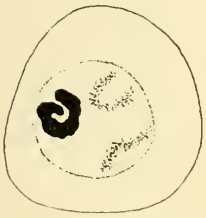

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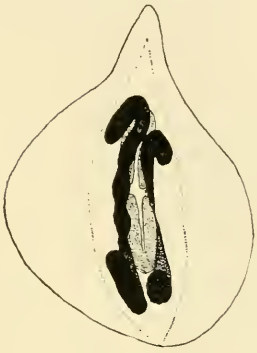

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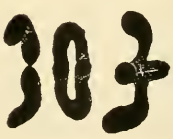

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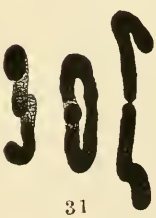

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Description of Figures

Fig. 32 Metaphase group in outline. Ac-c.

Fig. 33 Exceptional group of three rings. Ac-c.

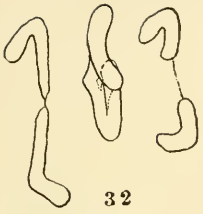
Figs. 34 and 35 Late prophase or early metaphase, from sections showing a single chromosome pair.

Fig. 36 Anaphase, showing the ends of one pair still overlapping and attached. Ac-c.

Figs 37–41 Exceptional cases of fragmentation and unequal division of the chromosome pair *a* of Fig. 23. Ac–c.

Fig. 42 Another case of irregular constriction of the same chromosome. Ac–c.

Fig. 43 Pair of second spermatocytes. Ac-c.



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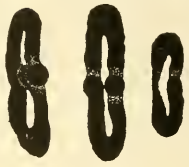

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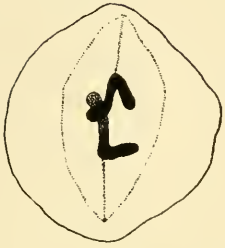

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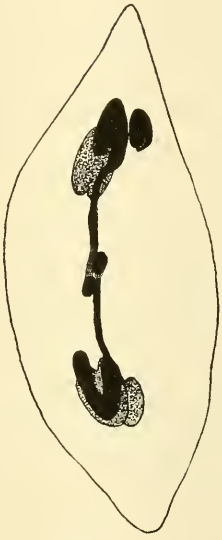

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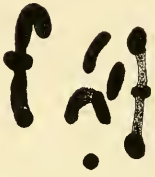

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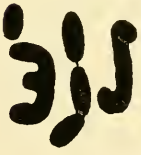

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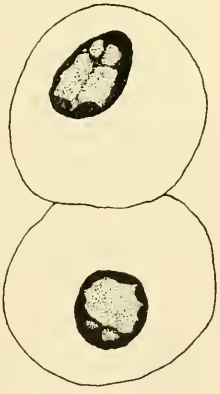

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Description of Figures

Fig. 44 Second spermatocyte; condition of the nucleus when the sister cells separate. Ac-c.

Fig. 45 Second spermatocyte; sister cells in later stage; *a* optical section through center of nucleus, *b* surface section of nucleus, chromosomes distributed over the nuclear membrane. Ac-c.

Fig. 46 Prophase of second maturation mitosis showing chromosomes already divided longitudinally Ac-c.

Fig. 47 Metaphase of second division,—chromosomes always tangled. Ac–c.

Figs. 48 and 49. Anaphases, Fig. 49 much flattened. Ac–c.

Fig. 50 Telophase.

Fig. 51 The smaller pair of chromosomes in prophase, *a* – *d* from sections of first spermatocytes, *e* from a spermatogonium.

Fig. 52 The smaller pair of chromosomes in metaphase and metakinesis of the first maturation mitosis, from sections.

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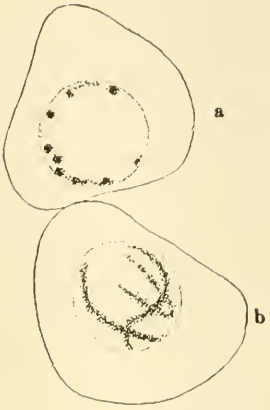

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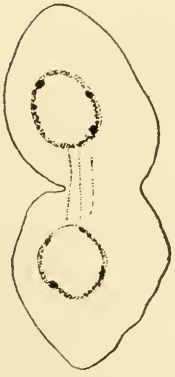

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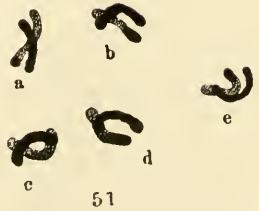

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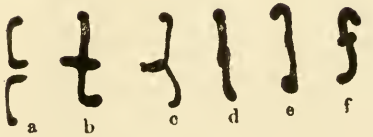

52

AN UNEQUAL PAIR OF HETEROCHROMOSOMES IN FORFICULA

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WITH FORTY-EIGHT FIGURES

In 1906, there appeared in the Zool. Anzeiger, vol. 30, no. 7, a preliminary paper entitled, "Die Spermatogenese von Forficula auricularia" by Herbert Zweiger, and the same year this author published in the Jena Zeitschrift, vol. 42, a more elabroate paper under the same title.

Zweiger found a variable number of chromosomes, 24 or 26 in the spermatogonia and 12 to 14 in the spermatocytes. He described a chromatin nucleolus in the growth stages of the spermatocytes; and in some of the first spermatocyte anaphases, a lagging pair of chromosomes which he calls "das accessorische Chromosom." He also states that in some cysts two such are found, making 14 in all. The numerical conditions were constant for each cyst, but not for all cysts of the same testis. In the second spermatocytes he found 12, 13 or 14 chromosomes.

Carnoy ('85) described the same species as having 10 to 14 chromosomes in the spermatocytes. La Valette St. George ('87) found 12 in first and 12 to 14 in second spermatocytes. Sinéty ('01) gives the numbers as 24 in spermatogonia and 12 in spermatocytes. These authors did not deal with the question whether or not heterochromosomes are present in Forficula.

Last summer while I was collecting material at the Marine Station in Helgoland during the third week in July, I chanced to find an abundance of Forficula and identified the insects with the aid of the laboratory collection, as Forficula auricularia. Having previously gone over the question as to heterochromosomes in

Anisolabis maritima, a related form, with Miss Randolph ('08) I was interested to see some preparations of Forficula, so I put up a number of testes in Gilson's mercuro-nitric fluid.

In September and early October I found what appeared to be the same species in Eisenach, Germany, and preserved more material. In November some of each set of material was embedded, sectioned and stained with thionin, at the Zoologisches Institut, Würzburg. On examining the preparations with the microscope, I was considerably surprised to find a perfectly clear case of an unequal pair of heterochromosomes in the first spermatocytes, and this is my excuse for adding another paper to the literature on the spermatogenesis of Forficula auricularia.

In the resting spermatogonia the chromosomes remain condensed, as figured by Zweiger, and are for the most part in contact with the nuclear membrane. Fig. 1 was drawn by focusing from the surface of the nuclear membrane down to about the center of the nucleus. In every case where favorable equatorial plates were found the number of chromosomes was 24. Figs. 2 and 3 were taken from different sections of the same testis, and Fig. 4 from another individual. Occasionally one finds a suggestion of a synizesis stage, but this stage is certainly very inconspicuous and probably very brief. Fig. 5 shows one such nucleus with one isolated chromosome which corresponds fairly well in size to the larger heterochromosome. There is no evidence as to when synapsis occurs.

At the beginning of the growth stage of the spermatocytes one finds the chromosomes passing from the concentrated condition of the spermatogonia (Fig. 1) through a transition stage (Figs. 6 and 7) into a spireme stage (Fig. 8), in which the chromatin thread is slender and pale in both thionin and iron-hæmatoxylin preparations (Fig. 8). The heterochromosome pair (x) is clearly distinguishable in these stages, and one, two or more plasmosomes are present (p).

The spireme soon shortens and thickens (Figs. 9–11) and frequently the heterochromosome pair may be seen to be composed of a larger and a smaller chromosome (Figs. 7 and 11). Fig. 12 shows other forms which the heterochromosome pair may assume

during these stages. Sometimes it contains a vacuole as in Fig. 10, especially in late growth stages. In the preparations stained with thionin the heterochromosomes can be distinguished from the plasmosomes with comparative ease, on account of their different staining qualities.

The spireme segments and splits longitudinally at about the same time (Fig. 13). Sometimes the daughter segments spread apart and twist as in Fig. 14, but they soon fuse again (Fig. 15) and most of the segments assume the form of loops, U's and twists (Figs. 15, 16, 17). Fig. 18 shows a variety of prophase forms. The twists and figure 8's are formed from such loops as are seen in Fig. 15, and further condensation gives the U and ring forms. The U-form is the most common (Fig 19), but rings and twists, and figure 8's with the ends twisted together are frequently seen. Figs. 20, 21, and 22 show transition stages from the rings and U's to the dumb-bell form in which the chromosomes come into the spindle. Occasionally one sees an incipient cross (Fig 21, *a*). Fig 22 shows a variety of intermediate and transition stages. Fig 23 is a tangential section of a nucleus in a late prophase, showing the heterochromosome pair (*x*) and three ordinary bivalents. Figs. 24 and 25 also show late prophase stages. In Fig. 25 the tetrad character of the bivalents is evident. In a spindle-prophase and metaphase, the tetrads are sometimes as clear as in Fig 26, both in thionin preparations and also in preparations where the differentiation of iron-hæmatoxylin has been carried to just the right point. The first spermatocyte metaphase usually looks like Fig. 27 from the Eisenach material, or Fig. 28 from the Helgoland collection, the heterochromosome pair being at one side of the plate and often remaining out of the plate longer than the other pairs (Fig. 28). Figs. 29 and 30 are earlier and later metaphase plates with the heterochromosome (*x*) distinguishable at one side of each group. In thionin preparations and in the paler iron-hæmatoxylin slides one finds some spindles in which the tetrad nature of some of the bivalents is shown both by longitudinal furrows and by the attachment of the spindle fibers to the split ends of the dumb-bells (Fig. 31). A similar stage seen from the pole of the spindle is shown in Fig. 32, some of the chromosomes showing the split. Fig. 33 gives

several views of the unequal pair in different stages of longitudinal splitting.

It will be seen from the figures and description given, that I find no evidence that the two halves of the bivalent chromosomes remain folded together parallel with each other, as described by Zweiger; but the rings and U's and V's all gradually straighten out so that the univalent elements of the dumb-bell-shaped chromosomes stand end to end in the spindle, and the bivalents often appear as typical tetrads.

Figs. 34 and 35 are early anaphases showing separation of the univalent components of both the unequal heterochromosome pair and the equal pairs. In my material the anaphase stages (Figs. 34, 35, 36) usually show nothing like Zweiger's "accessorisches Chromosom" (Zweiger, '06, Zool. Anz., Fig. 13; Jena Zeit., Fig. 25), but occasionally one finds it (Figs. 37 and 38). Fig. 37 is from the Eisenach material and Fig. 38 from a Helgoland preparation. The former preparation consisted of a single pair of testes in which it was possible to count the chromosomes in a number of spermatogonial plates: all had 24 (Figs. 2 and 3). There were also a large number of first spermatocyte plates, containing 12 chromosomes without exception. There was only one small cyst of second spermatocytes in metaphase; 12 chromosomes were counted in 24 cases, 13 in 2 and 11 in one. It is therefore evident that in this individual the lagging pair could not be an additional pair of accessory chromosomes making 26 for the spermatogonial number (Zweiger's explanation). Many pairs of daughter plates of the first spermatocytes were counted and 12 daughter chromosomes found in every case (Figs. 39 and 40). In these two pairs of daughter plates the heterochromosomes x_1, and x_2 were distinguishable.

In the second spermatocytes the usual number of chromosomes is 12 (Fig. 41 *b*), but in all testes in which this stage was at all abundant, there were occasional 11's (Fig. 41, *a*) and 13's (Fig. 41, *c–g*) in the same cysts with the 12's. Multipolar first spermatocyte spindles are sometimes seen, but they are not frequent enough to account for the irregular numbers in the second spermatocytes. Then, too, the numbers are always 11, 12 and 13 and there is no reason why multipolar mitoses should not give numbers both

larger and smaller. Therefore, although I have succeeded in finding very few cases of a lagging chromosome like that described by Zweiger and shown in Figs. 37 and 38, I am inclined to connect the irregular numbers in second spermatocytes with this chromosome, which, judging from its size, and from the behavior of the supernumerary heterochromosomes in Diabrotica soror and Diabrotica 12-punctata (Stevens '08), I think must be a precocious division of the smaller heterochromosome, x_2. In Fig. 41, *e*, two of the 13 chromosomes are unusually small, lie side by side, and were paler blue than the other chromosomes, a difference which is often noticeable between the larger (x_1) and smaller (x_2) heterochromosomes in metaphase of the first maturation mitosis in preparations stained with thionin. In this case the number, 13, may be due to precocious division of x_2 in the daughter cell to which it passed in the first mitosis. In such cases as are figured in Figs. 37 and 38, if one daughter element of the lagging chromosome goes to each second spermatocyte the numbers should be 12 and 13 with one smaller chromosome in each cell. In Figs. 41, *d*, *f*, and *g*, one of the 13 chromosomes is unusually small (*s*). The number 11 is more difficult to account for, unless the two heterochromosomes sometimes go to the same daughter cell; of this I have seen no evidence. In two cases I found that one chromosome which was out of the equatorial plane had been removed in another section, leaving 11, and in a few spindles seen from the side one chromosome has been considerably out of the equatorial plane. This may account for all of the 11's. All of the figures of 13's (Figs. 41, *e–g*) were cases where metakinesis had not begun, and it was perfectly certain that 13 distinct chromosomes were present. The chromosomes usually divide regularly as in Fig. 42 and give daughter plates containing 12 chromosomes as in Fig. 43, but occasionally one sees a lagging chromosome (Fig. 44) which in some cases is pulled out somewhat irregularly between the two groups of fused chromosomes as though dividing (Fig. 45), but is more commonly plainly included in one of the spermatids without being divided (Fig. 46). In fact, I have found no case where this chromosome was clearly divided as is the case with the lagging chromosome of the first division (Fig. 37, x_2). This lagging chromosome of the second

maturation mitosis is always paler than the polar mass of fused chromosomes, and is apparently about equal in bulk to one of the two lagging elements seen in Fig. 37. I should therefore think that the most probable interpretation of the irregular number of chromosomes in the second spermatocytes (13) and of the two lagging chromosomes is that the first (Figs. 37 and 38) is a precocious division of the smaller heterochromosome (x_2), and that the second (Figs. 44–46) is one of the products of such a division, already univalent and therefore not to be expected to divide again. In my material the irregularities in division and in numbers are comparatively infrequent. As the number 24 has always been found in the spermatogonia and 12 in the first spermatocytes, it would seem that only the spermatids which are the result of two regular divisions and therefore contain 12 chromosomes can become functional. The occasional cases of a lagging chromosome in the first spermatocyte mitosis suggest that the smaller heterochromosome in Forficula is in somewhat the same uncertain condition as to its behavior in maturation as is the case with the supernumerary heterochromosomes in the Diabroticas, which divide sometimes late in the first spermatocyte division and sometimes in the second division, thus giving rise to irregular numbers.

The heterochromosomes of two sizes can be distinguished in the spermatids as shown in Figs. 47, *a* and *b* and Figs. 48, *a* and *b*, each pair from one section of the same cyst.

DISCUSSION

In respect to the unequal heterochromosome pair, which judging from analogy with other insects, is probably to be associated with the determination of sex, my results differ from those of all others who have worked on the spermatogenesis of Forficula auricularia. Either the inequality in this pair of chromosomes has been overlooked, or the species is variable as to the character of its heterochromosomes in different localities. I thought at first that the difference in size of the heterochromosomes (x_1, and x_2) in the Helgoland material was somewhat more conspicuous than in that collected in Eisenach, but on further examination the dif-

ference between the two lots of material proved to be slight, if indeed there be any difference. The small heterochromosome is often flattened so that it looks smaller in side than in face view (Figs. 28 (H), 35 (H), 24 (E), 27 (E), 31 (E), 33 (E)). In either material the inequality in size of the components of this pair is conspicuous enough so that it is difficult to understand how anyone who was looking for heterochromosomes could overlook it.

As to the method of formation of tetrads I should disagree with Zweiger and agree with Sinéty in finding them formed in a manner typical for many insects, by transverse division and longitudinal splitting of the telosynaptic pairs of bivalents.

The indications are either that Forficula auricularia must be variable as to number of chromosomes in different localities, or that it is a composite species made up of several small species differing in number and behavior of their chromosomes. According to the latter supposition which seems to me the more probable, Sinéty's material with 24 and 12 chromosomes in spermatogonia and spermatocytes respectively, is a different small species from mine with usually 24 and 12 but sometimes 11 or 13 in the second spermatocytes, and both are different from Zweiger's which has 24, 26 or 28 in spermatogonia and 12, 13 or 14 in the spermatocytes. The peculiarity about Zweiger's numbers that I am unable to understand, is his finding the number of chromosomes different in first spermatocyte cysts of the same testis. I have always found the number in the first spermatocytes of insects constant for the individual. In cases like Diabrotica soror and D. 12-punctata, I get variable numbers in the spermatogonia and first spermatocytes of different individuals, but for each individual the spermatogonial and first spermatocyte numbers are constant while the second spermatocyte number is variable, as I find it in Forficula.

SUMMARY

1 In my material of Forficula auricularia, collected in Helgoland and Eisenach, Germany, I find 24 chromosomes in the spermatogonia, 12 in first spermatocytes, and usually 12 in second

spermatocytes and spermatids, though 11's and 13's are occasionally found in each individual.

2 An unequal pair of heterochromosomes is present in the first spermatocytes. The components of the unequal pair separate producing dimorphic second spermatocytes, spermatids and spermatozoa.

3 "Das accessorische Chromosom" of Zweiger appears to me to be a precocious division of the smaller heterochromosome, giving rise to irregular numbers of chromosomes in the second spermatocytes.

Bryn Mawr College
January 10, 1910.

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Description of Figures

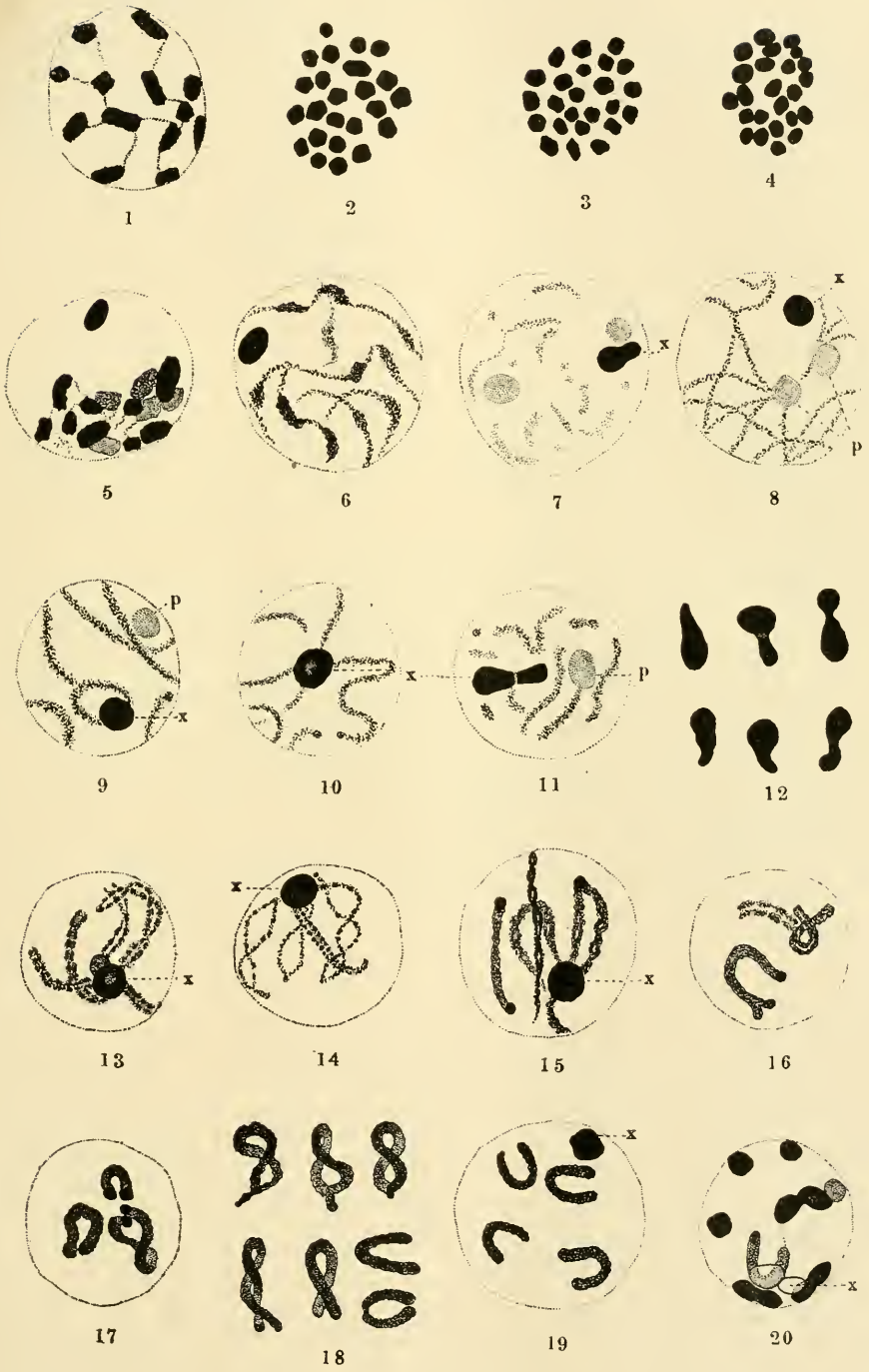
The figures were all drawn with camera lucida, Zeiss 1.5, oc. 12.

Lettering on figures

p = plasmosome.
x = heterochromosome pair.
x_1 = larger heterochromosome.
x_2 = smaller heterochromosome.
S = division product of x_2.

Fig. 1 Nucleus of resting spermatogonium. (H_1)
Figs. 2 and 3 Spermatogonial equatorial plates, 24 chromosomes. (E_1*)
Fig. 4 Spermatogonial equatorial plate, 24 chromosomes. (H_1*)
Fig. 5 Synizesis stage. (H_1)
Fig. 6 Transition stage from synizesis stage to spireme stage. (H_1)
Fig. 7 Later transition stage (E_2)
Fig. 8 Early growth stage, showing heterochromosome (x) and two plasmosomes. (H_1)
Fig. 9 Later growth stage. (H_1)
Fig. 10 Similar growth stage, showing vacuolated heterochromosome (x). (E_2)
Fig. 11 Growth stage showing the bivalent heterochromosome (x). (H_2)
Fig. 12 Various forms of the heterochromosome from same cysts as Fig. 7 and Fig. 11. (H_2 and E_2)
Fig. 13 Early prophase showing split segments. (E_2)
Fig. 14 Similar stage showing the daughter segments separated and twisted. (E_1)
Fig. 15 Later stage showing loop-form of segments. (E_1)
Fig. 16 Section of a nucleus containing three different prophase stages, split segments, V-shaped chromosomes, and twists. (E_2)
Fig. 17 Slightly later stage, showing U's and twists. (E_2)
Fig. 18 Various prophased forms, stage of Fig. 17. (E_2)
Fig. 19 Later stage, showing U-shaped chromosomes and the heterochromosome x. (H_1)
Fig. 20 Later stage, showing U's and dumb-bells, x in outline above the U-shaped chromosome. (H_1)

* H_1 and H_2 = Helgoland material; E_1 and E_2 = Eisenach material.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x
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x
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x
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x
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x
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x

Description of Figures

Fig. 21 Similar stage to Fig. 20, showing transition stages to dumb-bell form. (E_1)

Fig. 22. Various transition stages from same cyst as Fig. 21. (E_1)

Fig. 23 Tangential section of nucleus in dumb-bell stages, showing heterochromosome pair x. (H_1)

Fig. 24 Another dumb-bell stage. (E_2)

Fig. 25 Dumb-bell stage, showing tetrads. (E_1)

Fig. 26 Tetrads from spindle metaphase and prophase. (E_1)

Fig. 27 First spermatocyte spindle in metaphase. (E_2)

Fig. 28 Slightly earlier stage showing the unequal bivalent (x) out of the equatorial plate. (H_1)

Fig. 29 Early metaphase plate. (E_2)

Fig. 30 Later metaphase plate. (E_1)

Fig. 31 Metaphase, slightly later stage than Fig. 27, showing tetrad character of some of the bivalent chromosomes. (E_1)

Fig. 32 Equatorial plate showing longitudinal split in some of the chromosomes. (E_1)

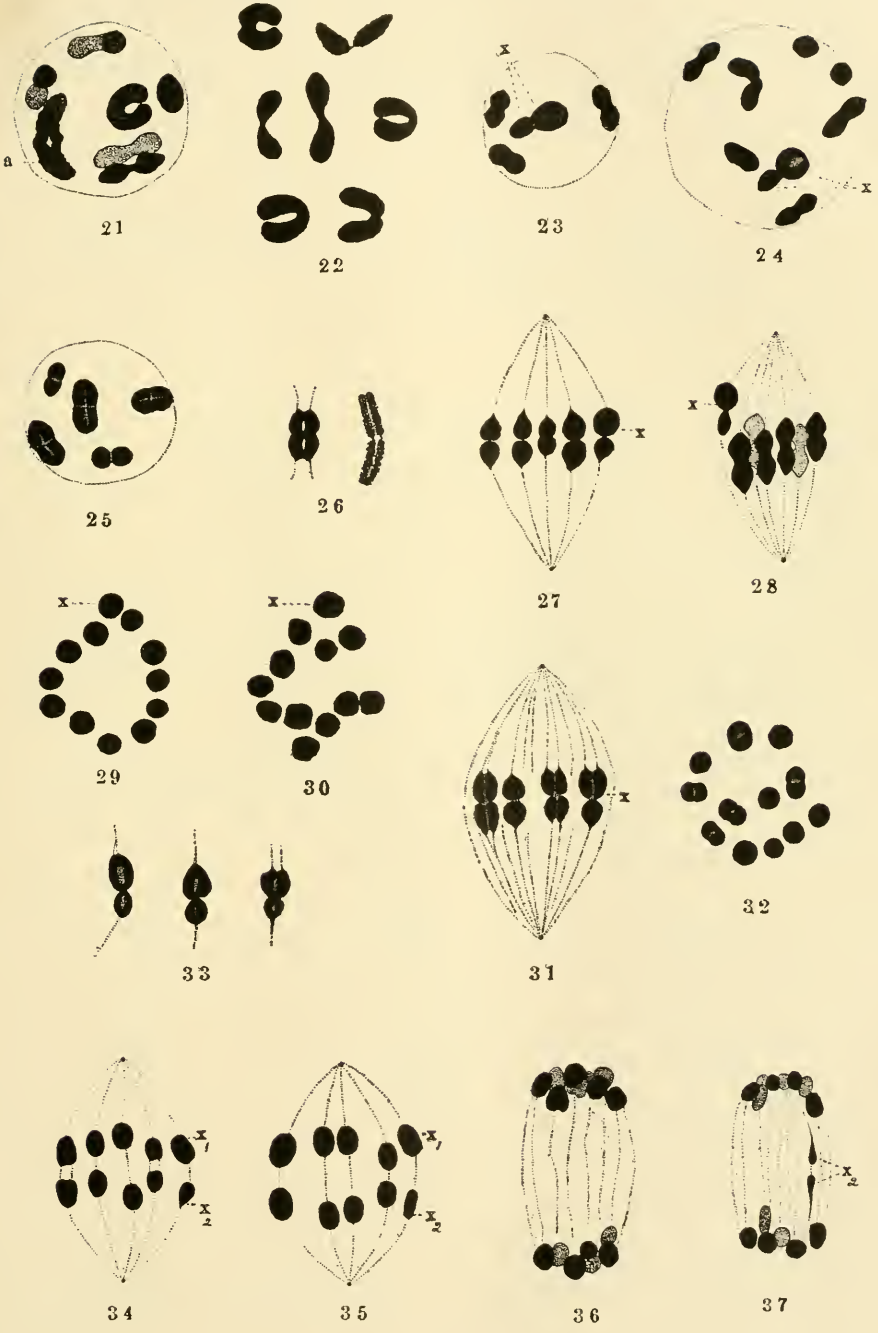
Fig. 33 The heterochromosome pair, showing split in both components. (E_1)

Fig. 34 Early anaphase. (H_1)

Fig. 35 Anaphase. (H_2)

Fig. 36 Late anaphase without the lagging chromosome. (E_1)

Fig. 37 Similar anaphase from same cyst as Fig. 36, with lagging chromosome (x_2). (E_1)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x_2
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x_1
x_2
35
36
x_2
37

Description of Figures

Fig. 38 Another anaphase, showing the lagging chromosome (x_2). (H_2)

Fig. 39 Daughter anaphase plates, x_1 and x_2 the heterochromosomes. (H_1)

Fig. 40 Daughter plates. (H_1)

Fig. 41 a–c. Second spermatocyte equatorial plates, containing 11, 12 and 13 chromosomes respectively. (H_1)

Fig. 31 d–g. Second spermatocyte equatorial plates. The smallest chromosome in *d*, *f* and *g* and the two small ones in *e* may mean precocious division of x_2. (E_2)

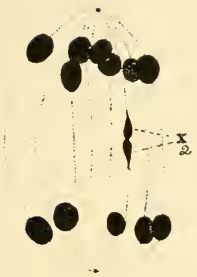
Fig. 42 Early anaphase of second mitosis. (E_2)

Fig. 43 Daughter anaphase plates of second mitosis. (H_2)

Fig. 44–46 Lagging chromosomes (*s*) occasionally seen in second maturation mitoses. (H_1)

Fig. 47*a* and *b* Young (spermatids from same cyst and same section (thionin staining), showing larger and smaller heterochromosome.) (H_1)

Fig. 48 *a* and *b*. Similar figures of older spermatids. -(H_1)



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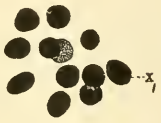

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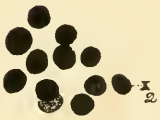

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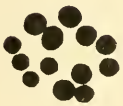

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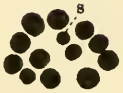

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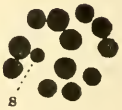

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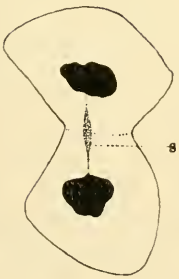

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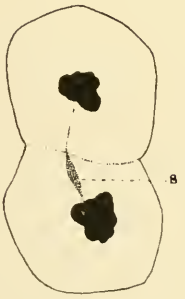

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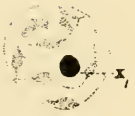

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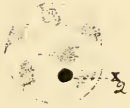

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, No. 207

A COMPARISON OF THE REACTIONS OF A SPECIES OF SURFACE ISOPOD WITH THOSE OF A SUBTERRANEAN SPECIES

PART I. EXPERIMENTS WITH LIGHT

A. M. BANTA

WITH SIX FIGURES

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INTRODUCTION

An investigation (Banta '07) of the natural history of the species of animals living within Mayfield's Cave near Bloomington, Ind., suggested the desirability of studying the reactions to various stimuli (light, etc.) of some cave species in comparison with the reactions to the same stimuli of near relatives living in other situations.

This study was undertaken in the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College. The first part, dealing with reactions to light, was carried out under the direction of Prof. E. L. Mark, to whom I am greatly indebted for providing exceptional facilities for conducting the research and for excellent suggestions and stimulating criticism.

Cave animals have long been a source of interest to men of science as well as to others. Their origin was long considered a matter of accident. Some animal, it was assumed, having wandered into a cave, or having been carried into it by a flood, was hardy enough to withstand the unusual conditions there, and, succeeding in finding a mate in a like straggler, was enabled to found a race. This race, as time went on, became more and more adapted to the unusual conditions and permanently established itself within the cave, ultimately producing a new and distinct species of cave animal. This "accident" hypothesis of the origin of cave life was well set forth and defended by Lankester ('93).

Eigenmann ('00, pp. 55–58), in discussing cave fishes, pointed out these objections to Lankester's hypothesis,—first that so many fishes of a single, extremely restricted small family should have "accidentally" become cave inhabitants, while no others in the same region (a region abounding in families and species of fresh-water fishes) became cave species; secondly the manifest impossibility of the survival of a species accidentally swept into a cave, unless it were already fitted for life in subterranean abodes; thirdly, that cave animals are negatively phototactic, a fact not to be harmonized with that part of Lankester's explanation which maintained that of those individuals which were accidentally swept into caves, the ones with the better eyes would follow the "glimmer of light and escape," leaving those with poorer eyes behind to become the progenitors of a blind cave race. Garman ('92, p. 240), Eigenmann ('90, and '00, p. 57), and the author (Banta '07, p. 98) have shown that animals undergo modifications suiting them for cave life in situations other than caves. In a former paper I ('07, p. 97) have laid stress upon the fact that cave animals belong to, and have originated within, families and genera which show a tendency to live in situations where the conditions resemble those of a cave, as regards darkness, moisture, etc.

It seemed desirable, therefore, to carry on a critical study of closely related species, one living within caves, the other outside of caves, subjecting both species to the same conditions and comparing their reactions. Such a study ought to show whether or not the cave species and its out-door relative are physiologically similar. So far as known to me, writers on cave species who have made any mention of the sense organs of these animals have, with one exception, noted in cave species, as compared with epigeal forms, better developed tactile organs, but less efficient organs of vision. But no extensive detailed observations on the relative sensitiveness to light and other stimuli of a cave animal and an outdoor animal of a similar sort had been made; such a comparison seemed worth making.

Among the many who have mentioned the better development of the tactile organs in subterranean animals as compensation for the loss of eyes may be mentioned the following: Packard ('88, pp. 123-130) reviewed the literature and cited many illustrations of this compensation, particularly in American cave animals. Hamann ('96) found the same to be true of European cave animals in general: likewise Chilton ('94, pp. 261–263) and Viré ('99) in discussing the subterranean animals of New Zealand and France, respectively, found evidence of this compensation. An exception to this theory of compensation is pointed out by Vejdovsky ('05, p. 12), who says that in Bathyonyx de Vismesi[1] Vejdovsky, from the depths of Lough Mask in Ireland, which has extremely degenerate eyes, the other sense organs of the head (Sinnespinsel und Sinneskapseln) are also less numerous and less well developed than in the common fresh-water amphipods.

As regards the isopods in particular, this increased development of other sense organs in compensation for the loss of eyes was noted by de Rougemont ('76) and subsequently by Leydig ('83, p. 36). Viré ('97, pp. 131–132) calls attention to a striking series of Asellidæ showing stages in the hypertrophy of these organs; first, the Asellus aquaticus which lives in brooks about Paris;

[1] This is a deep-water form, to be sure, but it deserves consideration in this connection, since the modifications of animals living in the depths of fresh water lakes are in general like those of subterranean animals.

secondly, those representatives of the species which live in the sewers of Paris, the latter having their tactile organs somewhat hypertrophied; thirdly, the representatives of the same species which live in the catacombs of Paris, these having the tactile organs still better developed, and, finally, the entirely blind subterranean Stenasellus Viréi, in which these organs are still further developed. The evidence seems to point to a considerably greater development of the tactile organs in cave species. It has been determined and is, indeed, a matter of common knowledge, that the blind fish (Amblyopsis) of the caves of the Ohio Valley is very sensitive to any disturbance in the water (c f. Packard '88, pp. 127-128), but, so far as I am aware, no attempt to make a comparative test of this increased sensitiveness to mechanical stimuli in an experimental way has been undertaken. An examination into the comparative physiology of the sense of touch in a cave species and in a nearly related surface species forms a part of my problem, and the results obtained will be set forth in a second paper.

Finally it was thought desirable to ascertain, if possible, what were the factors determining the relegation of one species to a cave, while a nearly related form did not betake itself to that habitat at all. This question received considerable attention. With this problem in mind, I sought in may cases the ultimate effects of various conditions with reference to their possible bearing on the determination of a cavernicolous or non-cavernicolous habitat, the detailed reactions of individual animals being then given only secondary attention.

MATERIAL

There are may cave animals which it is difficult to keep alive when they are removed from the caves, but the aquatic subterranean species, particularly the crustaceans, are readily kept in good condition if maintained in fairly clean water, moderately oxygenated, and not allowed to become too warm. Because of their availability and the ease with which they could be handled, the following two species were selected for comparison; the common subterranean isopod of the Ohio Valley, Cæcidotea stygia Packard, and the common and generally distributed fresh-water isopod,

Asellus communis Say. The latter occurs not only in the cave regions of the middle west, but also in the vicinity of Cambridge, Mass. The Cæcidotea stygia were obtained from Mayfield's cave near Bloomington, Ind., and from the caves on the Indiana University Experimental Farm at Mitchell, Ind. For collecting and forwarding much of this material I am indebted to the kindness of Drs. Charles Zeleny and W. L. Hahn of Indiana University.

Cæcidotea stygia Packard is a white, eyeless species. It seems to occur rather generally in subterranean waters throughout the Ohio Valley (cf. Banta '07, pp. 76–77). It has been found in wells, in most of the caves of the Ohio Valley, and in tile drains in Illinois (Forbes '76, p. 13). I have found it also above ground near Bloomington, Ind., in a spring and its stream and likewise under leaves in a sheltered ravine. W. L. Hahn informs me that at Donaldson's Cave near Mitchell, Ind., it occurs under stones in the cave stream outside the mouth of the cave. When found outside of caves it has been taken from under stones or dead leaves in waters closely associated with subterranean waters. Within caves, "It is often found along the edge of the pools or in the shallow parts of the streams . . . . More usually, however, it is found under stones in the water . . . Cæcidotea stygia is a weak species. It can not swim and usually crawls very slowly. It is nearly helpless out of water, its weak legs being scarcely able to push it along" (Banta '07, p. 76).

Asellus communis Say is the common fresh-water isopod. It is distributed, according to Miss Richardson ('05, p. 420), who gives the localities by states, from Massachusetts and Pennsylvania on the east to Michigan, Illinois and Mississippi on the west. Near Cambridge it is extremely abundant in many ponds and small streams. It is a more active species than Cæcidotea, and is usually found on the substratum, under stones or among dead leaves or crawling about and burying itself in the loose débris scattered there. Sometimes, however, it is seen climbing about over Ceratophyllum or other water plants, though it is most abundant in the more secluded situations. Occasionally it appears where the current is fairly strong; more generally it is to be met in fairly quiet waters or even in stagnant pools.

Since Cæcidotea stygia and Asellus communis are so closely related, they are appropriate species for comparison. Packard in his comparison of the two species showed them to be much alike structurally, indeed, he ('88, pp. 29-33) supposed Cæcidotea stygia to have been derived from Asellus, but placed it in a distinct genus because of its lack of eyes and its more slender body and appendages. Miss Richardson ('05, p. 410) has made use of only the characters Packard had proposed as a basis for separating the two genera. Asellus occurs in the cave regions of Indiana and, at present at least, has the same opportunity to be a cave inhabitant that Cæcidotea has. Asellus communis, unlike Cæcidotea, is pigmented about as fully as most crustaceans. Its eyes consist of from 12 to 20 irregular facets compacted together.

There is not an obviously greater development of the tactile organs about the head of Cæcidotea than about that of Asellus. The much more slender and flattened body and the longer and more slender antennæ and legs of Cæcidotea, however, would apparently contribute to greater sensitiveness on its part.

I. HORIZONTAL ILLUMINATION

1. Methods and Apparatus

The experiments with light were carried on in the basement of the Museum of Comparative Zoölogy in a west room, which could be made dark or arranged to admit either diffuse daylight or direct sunlight as desired.

Most of the experiments were made with artificial light, during which of course daylight, as well as direct sunlight, was excluded from the room. A glass tank (compare Fig. 1, p. 250, for the arrangement of the whole apparatus) 51 cm. long, 22.6 cm. broad and 7.7 cm. deep, inside measurements, was used to confine the animals during experimentation. Its sides were of plate glass 5.8 mm. thick and its bottom was a removable sheet of glass 3 mm. thick with a ground upper surface. For convenience in making records of the experiments, the side walls of the tank were divided into six equal sections indicated by vertical lines. These sections are

hereafter referred to by numbers, 1 to 6. This served as a means for estimating the relative positions of the animals at any given instant. The records were made by counting and recording the number of individuals in each section of the tank at certain intervals during the experiment. The enumerations were made by observing from above, care being taken to prevent, or reduce to a minimum, the possible reflection of light from above, so that the records were obtained without in any way interfering with the course of the experiment. For convenience and safety in handling the glass tank (Fig. 1, *IT*) it was placed within a larger wooden tank (*OT*) with glass ends 63.5 cm. long and 30 cm. broad. Both tanks were filled with water to a depth of 3 cm.

During experiments with horizontal illumination there was used as a heat screen (*HS*) a rectangular glass jar, 31 cm. long, 20 cm. high and 8 cm. from front to back, filled with filtered water.

Different sources of illumination (*L*) were used: a 6-glower, 220-volt, Nernst lamp of 772 c.p., and for lower intensities, either a 19 c.p., an 8 c.p., a 5 c.p., or an 0.8 c.p. incandescent lamp. Variation of the distance between the lamp and the tank was also used as a means of regulating the intensity of illumination. Much of the time while experimenting with horizontal illumination two lamps of the same intensity were placed at opposite ends of the tank. By a switch device, one light could be turned off and the other on, thereby reversing the direction of illumination without disturbing the animals or interrupting the observations. Only one 6-glower Nernst lamp was available however, so that it had to be shifted when a change in the direction of the light was desired. Extraneous light was carefully excluded. The lamp used was placed inside a lamp container (*LC*) made from a piece of blackened sheet-iron bent so as to form a rectangular box with open ends. One of these open ends was kept covered with black cloth; the other, which was directed toward the tank, was fitted with an opaque screen (*S*) that had in its center a diaphragm of adjustable size. When the lamp container was placed at some distance from the tank, the rays of light passing to the tank were confined within a hollow blackened half-cylinder (*HC*) thus preventing the escape of light into the room.

The lamp container was sometimes placed quite near the heat screen, which was always about 5 cm. from the end of the outer tank. In such cases the interval between the lamp container and the tank was not enough to permit the use of the half-cylinder, but the space was carefully covered over with black cloth. When the half-cylinder was used, the interval between the light-container and the cylinder was likewise covered with black cloth (*CS'*), and in a similar manner the interval between the cylinder and the tank (*CS''*). At the end of the light-container nearer to the tank the size of the opening in the opaque screen (*S*) was regulated so as to allow approximately only such rays to enter the half-cylinder as would reach the tank directly, i.e., without being reflected from the sides of the cylinder. A vertically sliding screen at the near end of the tank was used to cut out all rays except those entering below the surface of the water.

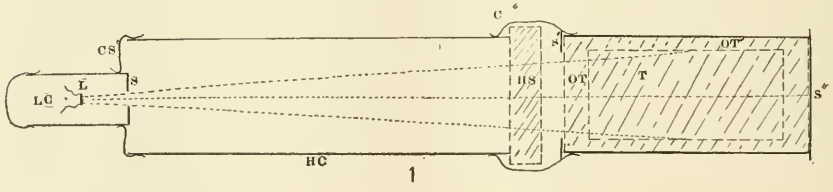

Fig. 1.

Fig. 1.—Diagram showing ground plan of apparatus used in experiments with horizontal illumination. *CS'*, *CS''*, cloth screens; *HC*, half-cylinder; *HS*, heat screen; *IT*, inner tank; *L*, source of illumination; *LC*, lamp container; *OT*, *OT'*, outer tank; *S*, screen with adjustable opening; *S'S''*, opaque screens.

The Asellidæ experimented with were ordinarily not very active, but after being handled and placed in new quarters they kept moving intermittently for some time. Hence they were generally allowed considerable time to become adjusted to their new surroundings before the experiments with light began. This was found desirable because otherwise thigmotactic or other stimuli resulting from the new conditions were for a time predominant, the light stimulus being at first so ineffective that the animals wandered about with apparent indifference to it. Sometimes the animals

to be experimented with were placed within a glass ring (17 cm. in diameter, located in the center of the inner tank) and allowed to settle there. This insured the settling of the animals in the beginning of the experiment in a neutral position, and facilitated the interpretation of their movements when subjected to light stimulation.

When all the conditions were favorable for the experiment, the glass ring was carefully lifted. It often happened that numbers of Asellus gathered in bunches at one edge or in one corner of the tank. The individuals were then very slow to leave the bunch, even under intense light stimulation, so that in such cases definite reactions were much delayed and sometimes did not appear at all. Bunches were less often formed when the animals were confined within the glass ring than when left free. Moreover, in removing the ring any aggregation formed at the angle between it and the floor of the tank was somewhat disturbed mechanically and the individuals composing the bunch were more quickly scattered than when the ring was not used. This mechanical stimulation lasted only a second and was wholly non-directive; consequently it in no way interferred with the influence of the light. The main advantage of the ring, however, was due to the retention of the animals in the middle of the tank, so that when they were subjected to light stimulation their movements were readily interpreted.

In the light experiments, as in all other experiments with Asellus and Cæcidotea, the same conditions were observed for both species, the two forms being studied one after the other in quick succession.

The relative inactivity and lack of responsiveness to light made it desirable to use a considerable number of individuals in each experiment. Although the numbers employed varied from 12 to 40, the most desirable number was found to be from 20 to 25. Because of the tendency of Asellus to collect in groups, and because of the thigmotactic responses of the species upon contact with one another, a great number of individuals were less responsive to light, and therefore unfavorable for experimentation. In the case of Cæcidotea, too, a larger number than 25 proved to be undesirable, as these animals are likewise very responsive to contact

with one another. Their responses, however, are of a different nature from those of Asellus. The Cæcidotea do not collect in bunches, but usually move away from one another very quickly at the slightest mutual contact. The thigmotactic response, then, is not, as with Asellus, positive, but on the contrary very decidedly negative.

Slight, non-essential modifications were made from time to time in the apparatus described above in order the better to fit it for particular conditions of experimentation.

2. *Asellus*

A. Following Previous Exposure to Light

In considering the reactions of the two species to special light conditions after their previous exposure to diffuse daylight, Asellus will be discussed first, although it is to be borne in mind that the corresponding experiments with the two species were carried on in quick succession.

Asellus was found to be not very responsive to light, and during the earlier experiments seemed so capricious that little uniformity could be detected in its responses. However, with improved conditions of experimentation and with better knowledge of the actions of the species in general, the responses were ultimately found to be fairly definite and uniform.

To intensities below about 2.5 candle meters (C.M.), however, Asellus is not at all responsive. This conclusion is based on the results of a number of experiments. Table 1 shows the results of an experiment with an intensity approximately 1 C.M., produced under the following conditions: 5 c.p. incandescent lamp at 2.25 meters from the middle of the tank.

In this table and in the following ones, showing results of experiments with horizontal illumination, the same general plan in the arrangement of data has been followed. In the first column, at the left, are indicated the time at which the experiment started and also the epochs at which the various observations were made. The six succeeding columns at the right of this one show the numbers of individuals in each of the six sections of the tank at each

TABLE I

ASELLUS COMMUNIS (25 individuals)

January 15, 1907

Illumination: horizontal, 1 C.M.; lamp placed at Section-1 end

Previous exposure: diffuse daylight

TIME OF MAKING RECORDS	+ SECTIONS OF THE TANK —						MEAN AVERAGE POSITION		Change in mean average position between the first and the last observation
	1	2	3	4	5	6			
8:10	2	1	3	3	7	9	4.56		
8:14	4	2	2	1	5	11	4.36		
8:18	3	2	2	1	4	12	4.54	4.46	
8:20	4	2	1	1	4	12	4.46		
8:30	4	2	1	1	4	12	4.46		
8:32	4	2	1	1	5	11	4.58		
8:35	3	3	1	1	5	11	4.46	4.5	
8:45	3	3	1	1	5	11	4.46		
8:55	4	2	1	3	3	10	4.26		
9:00	5	3	1	2	3	10	4.22	4.24	
9:30	5	2	1	3	1	11	4.20		
3:20	5	2	2	2	2	12	4.20		
3:38	5	3	0	3	3	11	4.16	4.18	
3·57	3	3	2	2	3	12	4.40		
Averages for the entire period.	3.9	2.3	1.4	1.8	3.8	11.7	4.38		+0.16

observation, the eighth column gives the mean average position at each epoch and finally, at the bottom of the last column, the change in the mean average position between the first and the last observation. The mean average position was calculated by multiplying the number of each section of the tank by the number of individuals in that section and dividing the sum of these products by the whole number of individuals.

The results of this experiment are graphically represented in Fig. 2, which shows a curve constructed by using the mean average positions as ordinates and the fifteen minute periods of the experiment as abscissas.

For convenience not more than one ordinate was used for each fifteen minute period of the experiment. If more than one record had been made during the fifteen minutes, the average of the mean

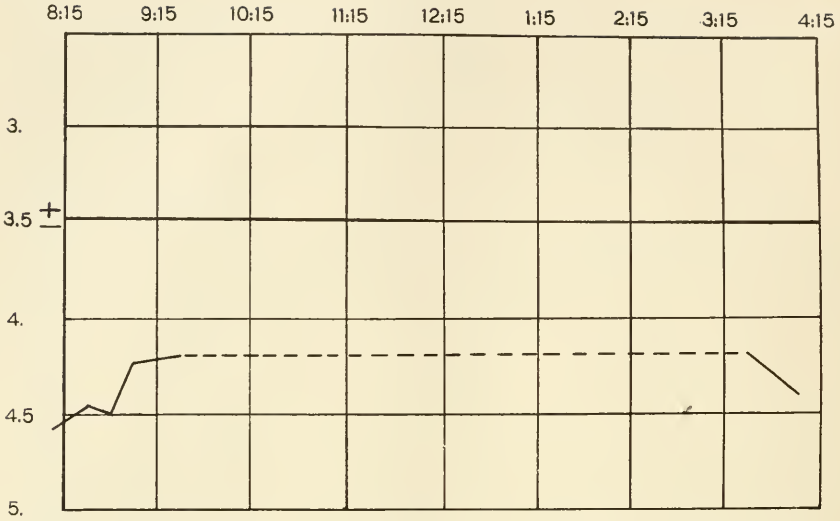

FIG. 2. Asellus communis (25 individuals); Jan. 15, 1907; previous exposure, diffuse daylight; illumination horizontal, 1. C. M.

average positions at the different epochs at which records were made was used, e.g., getting the mean average position for the period ending at 8:30 the average of the mean average positions for 8:14, 8:18, 8:20, and 8:30 was used. The base (heavy) line represents the middle of the tank. Points above the line indicate mean average position nearer the positive end of the tank and points below the base line indicate mean average position nearer the negative end of tank. In this experiment Asellus showed no response to the light stimulation. The mean average positions varied from a maximum of 4:56 at the start to a minimum of 4:16 at 3:38 o'clock, but at the close of the experiment, nearly 8 hours after the start, it was 4.40. Hence the change in mean average position from the beginning to the close of the experiment was only + 0.16. In view of the fact that when a definite light response is obtained with Asellus it is quite pronounced, this slight change in position is of no significance.

Numerous experiments were made upon Asellus with horizontal illumination at intensities ranging between 0.001 C.M. and 1.0 C.M.; the mean average position was as often changed in a negative as in a positive direction. This change was never sufficient

to be of any significance as far as the influence of light was concerned.

I expected to find a zone of fairly low light intensities in which the responses would be neutral, and below this a region of light intensities to which the animals would respond positively. But repeated experiments with low intensities did not reveal a single response in a positive direction.

Asellus, then, does not respond to light of the intensity of 1 C.M. or less. This is because the animal is not stimulated by light of such intensities or is stimulated so slightly as not to respond in a directive way.

The eye of Asellus is composed of from 12 to 20 more or less irregularly shaped facets and functionally is probably little more than a direction eye. It is situated somewhat mediad of the lateral margin of the head and slightly behind its anterior margin. Its location is, therefore, such that light from one side could not strike the eye on the opposite side, whereas light from above or from a strictly anterior or posterior direction would strike both eyes equally. If stimulated by light at all, it would seem as though the stimulus received from a small source of light ought to be directive in its effect. I incline to the opinion, however, that the animal is not at all *sensitive* to light of so low an intensity as 1 C.M. This opinion is further supported by the fact that Asellus, although positive to moderate and fairly low intensities, after being in the dark for several hours, is not *responsive* at all to intensities as low as 1 C.M.

Asellus after previous exposure to diffuse daylight, was negative to a light of 2.5 C.M. (19 c.p. incandescent at 2.75 m. from middle of tank), and to all greater intensities. The negative response was often slow in manifesting itself, but it occured with a fair degree of uniformity. Careful observations upon the actions of individual animals, as well as upon numbers of them at the same time, permitted the following analysis: Three different factors operated in producing the tardiness of the directive response. First, if the animals were once thoroughly settled in the tank before being exposed to the horizontal light, they were very slow to move, particularly if stimulated by light alone. This

apathy was often somewhat overcome by use of the glass ring already mentioned. The ring served to retain the animals in the center of the tank until settled, so that their movements could be readily interpreted with reference to the light effect, and at the same time by its removal the animals were more or less disturbed mechanically. Once roused from their inactive state, they responded more quickly in a directive way than they would have done if influenced by light alone. The mechanical stimulation produced by lifting the ring lasted only a second and was wholly non-directive. Secondly, the animals, if not yet settled after being transferred to the tank, responded to other stimuli (thigmotactic, etc.), which were powerful though non-directive in effect, so strongly that the directive influence of the light was not at once observable. After a time these non-directive stimuli became less influential in their effects, and the light with its directive influence became the effective stimulus. Thirdly, the photokinetic response to light tended for a time to mask the phototactic response.

The first effect of light of moderate and high intensities was often largely photokinetic, some of the animals starting up quickly very much as when mechanically stimulated. Sometimes, if the animals were already pretty thoroughly settled in the tank, no movements would occur for from 2 to 5 minutes, but usually after a period of 2 to 20 minutes, if the illumination were strong, a fairly general activity commenced. This activity, however, did not always manifest itelf at first as a directive response to light stimulation. The directive response, as indicated by the positions of the animals in the tank, ordinarily did not appear before an exposure varying from 15 to 90 minutes.

Observations of individual animals, however, brought out the fact that very often the phototactic response on the part of each individual occured rather quickly; but the animals on reaching the negative end of the tank recoiled from it and wandered the greater part or all the way back to the opposite end of the tank. This wandering about tended to obscure the directive reaction until, after a time, the animals became more or less settled. It was then that the directive response became most marked, for the animals came to rest in regions near the negative end, and often half, or

even more, of the entire number experimented upon stopped in the extreme negative section of the tank.

Hence, sometimes the activity of Asellus in responding to the light was itself the real cause of the apparent tardiness of the directive response, as indicated by the mean average position of the animals. The directive (phototactic) effect of the light in conjunction with a vigorous photokinetic effect, served to direct the animals to the negative end of the tank, from which owing to the relatively stronger photokinetic influence, they recoiled and wandered about sufficiently to be pretty generally scattered. As the photokinetic effect became less pronounced, however, the phototactic effect became relatively more effective and negative phototaxis caused the animals to congregate toward the end of the tank farther from the source of illumination.

In some cases the photokinetic influence was a very important factor during the first part of the experiment. The length of the tank (51 cm.) was sufficient to reduce this factor somewhat. At any rate, the ultimate response could not be affected in cases where the phototactic response was not altered by the length of exposure. In the present series of experiments the phototactic response did not change with long exposure to light. The photokinetic influence in its disturbing effect upon the phototactic responses will be seen to have been similar to the thigmotactic and other influences mentioned before, which kept the animals intermittently on the move for some time after they were introduced into the tank. It was only when all these non-directive influences had become subsidiary in their effect that the phototactic responses were recognizable and decisive.

The photokinetic effect naturally varied with the intensity, but the negative phototaxis also varied with the intensity, so that a directive response occurred as quickly with high as with the lower intensities.

Two experiments, in which Asellus after previous exposure to diffuse daylight was subjected to intensities of 3 C.M. (5 c.p. incandescent at 1.3 m. from middle of tank) and 2855 C.M. (772 c.p. 6-glower Nernst lamp at 0.52 m. from middle of tank) are given in detail in Tables II and III.

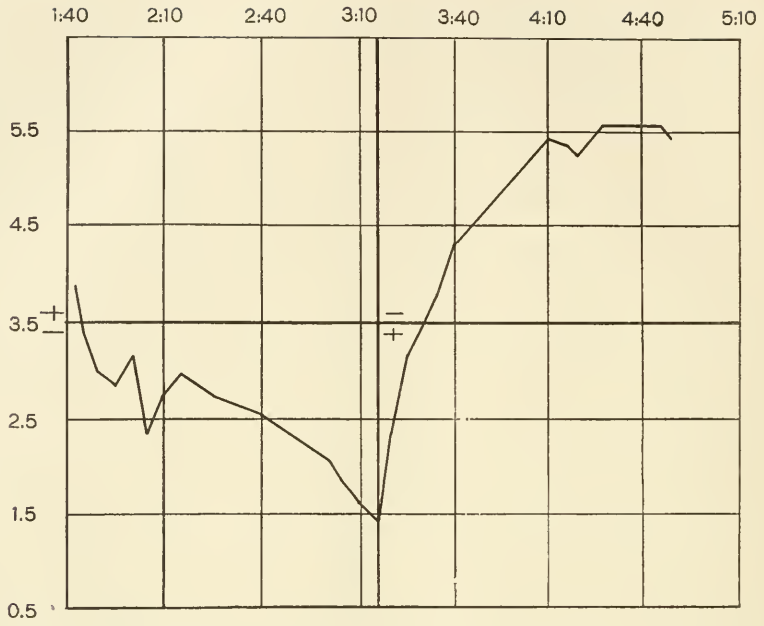

Fig. 3. Asellus communis (22 individuals); Nov. 24, 1906; previous exposure, diffuse daylight; illumination horizontal, 2855 C. M.

A graphic representation of the experiment recorded in Table II is given in Fig. 3. The method of representation is the same as explained in connection with Fig. 2 (p. 254).

Reference to Table II or Fig. 3 shows that in this experiment Asellus after exposure to diffuse daylight was decidedly negative to light of an intensity of 2855 C.M. These animals had been in the tank but 10 minutes when the experiment began, hence, the thigmotactic influence was still quite effective. The mean average position at the start, 1:43 p.m., was 3:86; after five minutes exposure, at 1:48, it was 2:95; after fifteen minutes, at 1:58, 2:64, with a tendency to collect at the negative end already beginning to manifest itself. But the phototactic response was still not the most obvious one, for while at 2:07 p.m., (twenty-four minutes after the experiment began) the mean average position was 1.95, and 13 indivicuals were in Section 1, four minutes later the mean position was 3.55 and only 8 were in Section 1, an equal number

being in Section 6. This shows that the non-directive thigmotactic, or other, influences due to the transference of the animals to new quarters, and the photokinetic influences were still effective. However, after about an hour from the beginning of the experiment the collecting toward the negative end and the avoiding of the positive end became quite marked, and little tendency to move toward the positive end manifested itself. The animals

TABLE II

ASELLUS COMMUNIS (22 individuals)

November 24, 1906

Previous exposure: diffuse daylight

Illumination: horizontal, 2855 C.M.; lamp at Section-6 end

In tank 10 minutes

TIME OF MAKING RECORDS	− SECTIONS OF TANK					+	MEAN AVERAGE POSITION		
	1	2	3	4	5	6			
1:43	4	0	2	8	5	3	3.86		
1:44	6	1	4	7	2	2	3.18		
1:45	4	4	2	7	1	4	3.41	3.35	
1:46	5	1	4	7	1	4	3.45		
1:48	5	4	4	6	2	1	2.95		
1:50	8	0	5	5	0	4	3.05	3	
1:52	8	2	3	2	3	4	3.09		
1:58	12	2	1	1	1	5	2.64	2.86	
2:00	9	3	3	0	1	6	2.95		
2:03	7	4	0	3	1	7	3.36	3.15	
2:05	10	3	4	3	1	1	2.32		
2:07	13	3	2	3	0	1	1.95		
2:11	8	1	2	1	2	8	3.55	2.75	
2:13	8	3	2	1	2	6	3.18		
2:15	10	1	4	2	1	4	2.77	2.97	
2:25	12	1	1	0	5	3	2.73		
2:40	10	2	3	4	1	2	2.54		
2:52	11	3	3	2	2	1	2.27		
3:00	9	5	5	3	0	0	2.09		
3:03	12	5	2	0	3	0	1.95		
3:05	13	4	4	0	0	1	1.77	1.86	
3:08	13	6	2	0	0	1	1.68		
3:10	16	3	2	0	0	1	1.54	1.61	
3:15	18	2	1	0	0	1	1.41		

Change in mean average position between the first and the last observation

−2.45

TABLE II—Continued.

Lamp changed to section-1 end of tank

TIME OF MAKING RECORDS	+ SECTIONS OF TANK					—	MEAN AVERAGE POSITION		Change in mean average position between the first and last observation
	1	2	3	4	5	6			
3:18	14	3	1	2	1	1	1.91		
3:18½	12	4	1	1	3	1	2.18	2.34	
3:20	9	6	2	0	2	3	2.5		
3:21*	7	5	5	0	0	5	2.82		
3:22	3	11	1	2	1	4	2.95		
3:23	5	8	2	2	1	4	2.91	3.16	
3:24	3	9	3	0	1	6	3.23		
3:25	2	8	0	0	6	7	3.91		
3:26	2	6	5	0	3	6	3.54		
3:27	0	6	5	4	1	5	3.71		
3:28	0	9	4	4	1	4	3.45	3.45	
3:29†	3	5	5	4	3	2	3.23		
3:30	3	5	5	3	3	3	3.32		
3:33	2	5	5	4	1	5	3.55	3.8	
3:35	1	5	4	2	2	8	4.05		
3:37	0	5	3	2	4	8	4.45	4.31	
3:40‡	2	4	2	3	2	9	4.18		
4:10	0	1	1	1	4	15	5.41		
4:15	0	1	1	2	3	15	5.36		
4:19	0	2	1	1	4	14	5.23		
4:27	0	1	1	0	3	17	5.55		
4:31	0	1	1	0	3	17	5.55		
4:45	0	1	1	0	3	17	5.55		
4:48	0	1	1	0	5	15	5.45		−3.54

*General movement at sections 1 and 2.

†A recoil from section-6 end, as at this time more individuals were moving from than toward the section-6 end of tank.

‡Some individuals still wandering about.

seemed fairly well settled at 3:15 p.m., an hour and thirty-two minutes after the experiment was begun. All but one were in the negative half of the tank and 18 were in Section 1, while the mean average position was 1.41; a change in a negative direction of 2.45 since the beginning of the experiment.

At this juncture the light was changed to the opposite end of the tank and a record of positions made as soon as possible after the change. The animals began to respond to the light stimulus

quickly and a very general and consistent migration toward the negative end continued for about seven minutes, when the photokinetic effect became apparent in the turning back of many individuals upon reaching the negative end of the tank. At 3:29 p.m., more seemed to be moving toward the positive than toward the negative end. These movements were clearly due to photokinesis, for the animals had by this time been in the tank long enough to have become thoroughly adjusted to it. At 3:40, 22 minutes after the position of the light was changed, considerable wandering about in the tank was noticeable, but 51 minutes after changing the light, at 4:09, the animals seemed pretty well confined to the negative end, though wandering about there to some extent. After 4:27 the movements were slight. During the $1\frac{1}{2}$ hours following the reversal of the direction of the light, the mean average position changed from 1.91 to 5.45, representing an average movement of 3.54 in a negative direction.

The above experiment is typical for the reactions of Asellus. The phototactic influence is often slower in asserting itself than in the experiment here recorded, but the other influences appear in this experiment in a characteristic way. A number of experiments with the same intensity yielded similar results, bearing out the conclusion that Asellus is decidedly negative to such an intensity after previously being in diffuse daylight.

The results of another experiment with Asellus following exposure to diffuse daylight are given in Table III, the intensity of the light in this case being only 3 C.M. (19 c.p. incandescent at 2.75 m. from middle of tank.)

As these animals had been in the tank in diffuse daylight within the glass ring for 55 minutes before the experiment began, they seemed fairly well settled and were near the center of the tank. While they were not very active at the time the ring was removed, yet, with freedom to move in any direction, they responded very promptly to the directive light. The response was so prompt and the movements so general that at first accurate counts could not be made. A part of this movement was due to photokinesis, rather than phototaxis, for the number and position of those in the positive end varied considerably. Whereas two or three min-

TABLE III

ASELLUS COMMUNIS (36 individuals)

February 23, 1906

Previous exposure: diffuse daylight

Illumination: horizontal, 3 C.M.; lamp at Section-6 end

In tank within glass ring 55 minutes before experiment began

TIME OF MAKING RECORDS	− SECTIONS OF TANK					+	MEAN AVERAGE POSITION	Change in mean average position between the first and the last observation
	1	2	3	4	5	6		
10:50	0	0	18	18	0	0	3.50	
10:51	10	12	5	2	3	4	2.67	
10:52	12	9	5	0	7	3	2.72	
10:53	14	5	9	5	2	1	2.42	
10:54	18	8	3	2	4	1	2.14	
10:56	14	8	7	3	1	3	2.39	
10:58	15	5	6	3	4	3	2.58	
11:00	14	5	4	2	6	5	2.89	
11:05	16	6	5	2	1	6	2.56	
11:41	11	7	4	3	8	3	2.94	
11:44	10	6	2	8	3	7	3.25	
12:23	16	6	5	3	0	6	2.53	
2:25	17	4	1	4	5	5	2.75	
2:35	11	7	3	1	3	11	3.31	
3:45	15	7	3	5	2	4	2.55	
5:05	20	6	2	3	1	4	2.19	−1.31

utes after the light was turned on only 8 or 10 individuals were in the positive end of the tank, at 11:44, nearly an hour after the experiment began, 18 were in the positive half and their positions were very different from what they had been three minutes before (11:41), when 14 were in the positive half of the tank.

This general activity was very pronounced at 11:05, 15 minutes after the experiment began. At 11:41 I made the following note in my record book "quite active and apparently a general movement toward + is beginning." This apparent general movement was due largely to the photokinetic activity, which at this particular time happened to produce a general movement in a positive direction. At 12:23 the activity was still very marked, but at 2:25 it was much less pronounced, and at 3:45 and 5:05 there were only slight movements.

The experiments described above serve to illustrate the series in which Asellus after exposure to diffuse daylight was subjected to illumination with light of various intensities from 2.5 C.M. to 2855 C.M. Many of the reactions were less pronounced than those described above, and sometimes the results were not very definite. Generally such cases were readily explicable as due to the apathy of the animals after they had once become thoroughly settled in the tank; for such indefinite results usually came from testing animals which had been in the tank 24 hours or longer.

From the experiments with Asellus communis when subjected to light of various intensities after previous exposure to diffuse daylight, the following conclusions are drawn:

1 Asellus does not respond to light below about 2.5 C.M. intensity, but responds to light from 2.5 C.M. to 2855 C.M. intensity.

2 This response consists of two factors, a non-directive photokinetic effect and a directive negative phototactic effect. The former is often the prevailing influence at the start, but the phototactic influence becomes the effective one as the photokinetic effect decreases and eventually disappears.

B. After being in Darkness

In conducting experiments on Asellus which had been kept in darkness before the beginning of observations, the following method was pursued. The animals were first placed in the tank and left in the darkened room. In addition to darkening the room, the outer tank was covered with light-proof screens. After the animals had been thus kept in the dark for the desired length of time, the screens were carefully removed and the lamp, which had previously been placed in position for the experiment, was suddenly made light.

Mention has already been made of the fact that Asellus does not respond to light intensities of 1 C.M. or less. The statement holds true whether the Asellus has been previously exposed to light or has been in the dark. Several intensities between 0.001 C.M. and 1 C.M. were tried, but definite responses were not

obtained. This lack of response to light of 1 C.M. or less (being found in animals after their retention in darkness when their response is normally positive, as well as after their exposure to light when their response is normally negative) may be taken as evidence that Asellus is *not at all sensitive* to light of such intensities. The range of intensities to which Asellus failed to respond was, as far as the evidence went, the same for animals previously in darkness and those previously in light.

After retention in darkness for several hours, Asellus gives a positive response to light of intensities between 2.5 C.M. and 80 C.M. (19 c.p. incandescent at 0.49 m. from middle of tank).

TABLE IV

ASELLUS COMMUNIS (16 individuals)
January 13, 1906
Previous exposure: darkness (in tank) for 40 hours
Illumination: horizontal, 2.5 C. M.; lamp at Section-1 end

TIME OF MAKING RECORDS	+ SECTIONS OF TANK					—	MEAN AVERAGE POSITIONS
	1	2	3	4	5	6	
8:19	4	4	0	2	2	4	3.38
8:20	4	2	1	1	3	4	3.62
8.21	4	3	0	2	3	3	3.4
8:22	5	1	2	3	2	2	3.13
8:23	6	2	1	4	0	2	2.73
8:24	8	1	4	0	1	2	2.44
8:25	7	1	3	0	2	2	2.47
8:26	7	1	4	0	1	2	2.53
8:27	8	1	4	0	1	2	2.44
8:28	8	1	4	0	1	2	2.44
8:29	8	1	4	0	1	2	2.44
8:30	8	1	4	0	1	2	2.44
8:31	8	1	2	1	1	2	2.47
8:32	7	1	4	0	1	2	2.53
8:33	7	2	3	1	0	3	2.62
8:34	8	1	4	0	0	3	2.5
8:35	8	3	2	0	1	2	2.31
8:36	9	2	1	1	1	2	2.31
8:37	9	2	1	1	1	2	2.31
9:05	9	2	2	1	0	2	2.19
9:32	9	3	1	1	0	2	2.13

Change in mean average position between the first and the last observation +1.25

TABLE V

ASELLUS COMMUNIS (18 individuals)
December 12, 1906
Previous exposure: diffuse daylight
Illumination: horizontal, 80 C. M.; lamp at Section-6 end
Experiment began as soon as animals were in tank

TIME OF MAKING RECORDS	+ SECTIONS OF TANK					—	MEAN AVERAGE POSITIONS	Change in mean average position between the first and the last observation
	1	2	3	4	5	6		
2:40	0	0	9	9	0	0	3.5	
2:41	4	5	5	4	0	0	2.5	
2:42	11	3	2	1	0	1	1.83	
2:43	8	4	3	0	2	1	2.28	
2:44	6	4	2	1	1	4	2.94	
2:45	10	2	0	0	1	5	2.72	
2:46	11	2	1	0	1	3	2.28	
2:47	6	2	4	3	0	3	2.89	
2:48	5	3	3	1	1	5	3.28	
2:49	3	2	3	4	2	4	3.67	
:50	3	2	4	4	2	3	3.5	
2:51	9	2	2	1	2	2	2.5	
2:52	7	5	1	1	2	2	2.56	
2:53	12	0	0	0	3	3	2.5	
								—1.0

Satisfactory positive reactions were obtained from Asellus after it had been kept in darkness for three or four hours or longer. Often when the intensity was only 2.5 C.M. the positive response was not very evident. The most marked positive response followed exposure to intensities between about 11 C.M. (19 c.p. incandescent at 1.32 m. from middle of tank) and 80 C.M. Asellus exposed to an intensity of 2855 C.M. often appeared positive at first, but remained so for only a very short time, then becoming negative.

Table IV shows the results of an experiment with 16 Asellus when subjected to light of 2.5 C.M. intensity after retention in darkness for 40 hours. This Table shows that the animals began to respond to the directive light within three or four minutes and that within sixteen minutes virtually the maximum reaction had taken place. Few random movements occurred in this experi-

ment, hence little photokinetic effect was to be inferred. The reaction was clearly positive and continued positive until the close of the experiment, although in another experiment the same individuals *following their exposure to diffuse daylight* were negative to the same intensity of light.

Two additional experiments are here given to show by contrast the difference in the nature of the responses when the animals had previously been exposed to light and when they had been in darkness. December 12, 1905, 18 Asellus after exposure to diffuse daylight were placed in the tank and at once subjected to light of 80 C.M. intensity. Table V and the continuous line in Fig. 4 show the results of this experiment.

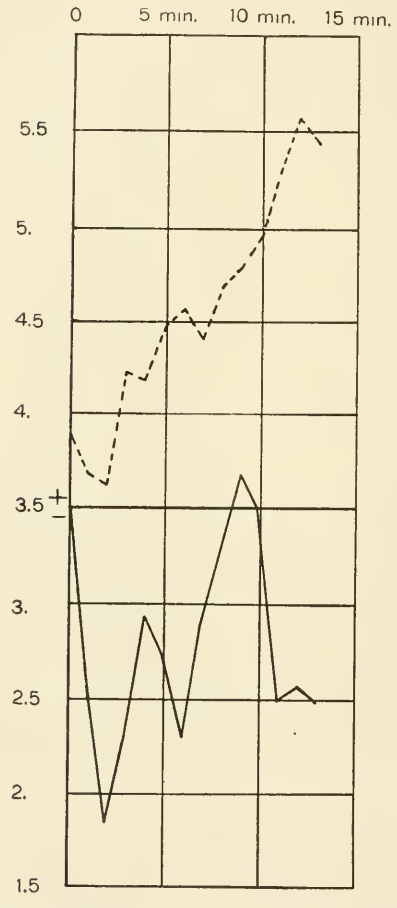

Fig. 4. Asellus communis (18 individuals). Graphic representation of results of experiments shown in Tables V and VI. Broken lines based on Table VI.

The experiment was continued for only thirteen minutes; the response was negative and very marked. Many of the individuals started up rather quickly, and all which responded at once moved away from the light. A little later two started toward the light. By that time, however, some had reached the negative end and were turning back. The record made at this time—two minutes after the start—shows the maximum response, as judged by the change in mean average position. It will be seen that the photokinetic effect in this experiment was very marked, but that, nevertheless, the positive phototaxis

was evident almost immediately. After the lapse of thirteen minutes, when the experiment was discontinued, the animals were closely covered and left in darkness until the following day.

The same animals were then experimented with under exactly the same conditions as on the previous day, except that in the meantime they had been in darkness for 16 hours, whereas on the day before they had previously been exposed for a long time to diffuse daylight. There was one other difference in the conditions of the two experiments, but it was non-essential; in the first case the animals were in the tank for only a short time before the experiment began, whereas in the second case they had been in the tank about 24 hours. Other experiments showed that this would, in any case, influence only the time at which the reaction occurred and that it would not affect the nature of the reaction.

Table VI and the broken line of Fig. 4 show the results of this experiment.

TABLE VI

ASELLUS COMMUNIS (18 individuals)
December 13, 1906
Previous exposure: darkness (in tank) for 16 hours
Illumination: horizontal, 80 C. M.; lamp at Section-6 end

TIME OF MAKING RECORDS	— SECTIONS OF TANK.					+	MEAN AVERAGE POSITIONS	Change in mean average position between the first and the last observation
	1	2	3	4	5	6		
11:15	6	1	0	1	2	8	3.89	
11:16	6	1	0	3	2	6	3.67	
11:17	4	3	1	2	4	4	3.61	
11:18	4	1	0	2	4	7	4.22	
11:19	4	1	1	3	0	9	4.17	
11:20	3	1	1	2	2	9	4.44	
11:21	3	1	0	3	1	10	4.56	
11:22	3	1	1	3	1	9	4.39	
11:23	3	1	0	0	5	9	4.67	
11:24	1	2	1	2	2	10	4.78	
11:25	0	2	1	3	2	10	4.94	
11:26	0	0	3	2	0	13	5.28	
11:27	0	0	1	2	1	14	5.56	
11:28	1	0	1	1	0	15	5.44	
								+1.55

The response was somewhat less prompt in the second experiment, but it was as definitely positive as the response in the first experiment was negative.

Numerous experiments were also made with other intensities (3 C.M., 11 C.M., and 25 C.M.). The results support the conclusion that Asellus after being kept in the dark is positive to all intensities of light between 2.5 C.M. and 80 C.M. Some of these experiments were continued for periods varying from 3 to 5 hours and the positive response persisted throughout the whole time. The maximum response, however, usually occurred between 30 and 90 minutes after the experiments began. When Asellus was subjected to a light intensity of as much as 2855 C.M. the animals very soon sought the negative end of the tank, although for a short time the response was often positive. These experiments indicate that after retention in darkness Asellus is positive even to light of fairly high intensities, but that with intensities as high as 2855 C.M. it very soon becomes negative.

From the foregoing experiments with Asellus when subjected to horizontal illumination of various intensities, after being in darkness, these conclusions have been reached:

1 Asellus does not respond to intensities of 1 C.M. or less.

2 It does respond to intensities from 2.5 C.M. to 2855 C.M.

3 This response is largely a direct phototactic effect, since the animals before exposure to specific intensities of light had been retained in darkness in the tank and of course had become thoroughly settled there; but the photokinetic element is also recognizable.

4 The immediate response to intensities from 2.5 C.M. to 80 C.M. is positive and continues to be so for at least 3 to 5 hours.

5 With an intensity of 2855 C.M. the response is often positive at first, but soon becomes negative.

By way of a general summary of the effect of horizontal illumination upon Asellus, it may be said, that after exposure to diffuse daylight it is negative to all intensities greater than about 2 C.M. It does not respond to lower intensities. Light has both a photokinetic and phototactic effect upon Asellus. The photokinetic effect is often sufficient to mask for a time the phototactic

influence, but this influence, even though in some cases it may not be asserted at once, ultimately appears, the photokinetic effect meanwhile becoming less pronounced.

After retention in darkness for a few hours, Asellus is positive to intensities between 2.5 C.M. and 80 C.M.; but to intensities of 1 C.M. or less it does not respond. To an intensity of 2855 C. M. Asellus is generally negative, though sometimes it is positive at first, but in that case it very quickly becomes negative.

3. *Cæcidotea*

The experiments upon Cæcidotea were made under precisely the same conditions as in the case of Asellus. Cæcidotea was found to be still less responsive to light than Asellus. Even after the conditions of experimentation and observation had been so refined that Asellus was seen to be definitely responsive to horizontal illumination, Cæcidotea long seemed quite unresponsive, except in a purely photokinetic way. Finally, however, numerous careful experiments showed that Cæcidotea was feebly responsive in a directive way to horizontal illumination. This species, like Asellus, after being transferred to the tank, kept up random movements for some time, doubtless as the result of thigmotactic or some other non-directive stimulus, and these random movements were even more marked and longer continued—on an average, nearly twice as long—than with Asellus.

The glass ring was often used to confine the Cæcidotea until they should become thoroughly settled in the middle of the tank.

Because of the general reluctance of the animals to move or to respond to light stimulation after they had once become settled, many of the experiments were begun as soon as the animals were placed in the tank. In such cases directive results from light stimulation ordinarily appeared only after the lapse of some time; for the movements due to thigmotaxis or other non-directive stimuli predominated at first.

With Cæcidotea, as with Asellus, the effects of stimulation by light *appeared to be* both photokinetic and phototactic. In many cases when animals almost or quite settled in the tank were sub-

TABLE VII

CÆCIDOTEA STYGIA (26 individuals)
November 7, 1906
Previous exposure: diffuse daylight
Illumination: horizontal, 2855 C. M.; lamp at Section −6 end
Records begun 5 minutes after animals were transferred to tank

TIME OF MAKING RECORDS	— SECTIONS OF TANK					+	MEAN AVERAGE POSITIONS
	1	2	3	4	5	6	
9:20	1	0	6	16	3	0	3.77
9:25	4	0	9	9	1	3	3.46
9:30	1	3	9	10	1	2	3.50
9:35	2	2	6	6	4	6	4.0
9:40	4	2	6	9	2	3	3.46
9:45	6	0	5	8	3	4	3.54
9:50	5	4	6	4	0	7	3.42
9:55	5	2	6	6	2	5	3.50
10:00	6	2	5	5	1	7	3.54
10:05	7	4	4	3	2	6	3.27
10:10	7	3	2	3	4	7	3.58
10:15	7	5	2	5	1	6	3.23
10:20	5	5	3	3	3	7	3.58
10:25	6	3	6	3	4	4	3.31
10:30	9	3	3	3	2	6	3.15
10:35	9	2	6	4	0	5	2.96
10:40	7	4	5	4	0	6	3.15
10:45	7	6	4	3	1	5	3.0
10:50	7	5	3	4	1	6	3.19
10:55	6	5	4	4	2	5	3.23
11:00	6	7	4	3	0	6	2.08
11:05	5	6	7	0	2	6	3.23
11:10	5	6	4	2	1	8	3.46
11:15	7	4	1	5	3	6	3.42
11:20	9	5	2	0	1	9	3.23
11:25	6	3	5	0	4	8	3.65
11:30	5	0	6	2	4	9	4.04
11:35	5	2	4	0	8	7	4.96
11:50	8	6	1	2	3	6	3.15
11:55	7	9	2	1	3	4	2.85
12:00	7	4	5	2	1	7	3.27
12:50	9	3	9	1	1	3	2.65
12:55	8	6	7	1	0	4	2.65
1:00	8	6	7	1	0	4	2.65
1:05	11	6	5	2	0	2	2.23
1:10	12	3	3	5	0	3	2.50

Change in mean average position between the first and the last observation

TABLE VII Continued.

TIME OF MAKING RECORDS	− SECTION OF TANK					+	MEAN AVERAGE POSITIONS	Change in mean average position between the first and last observation
	1	2	3	4	5	6		
2:30	13	0	9	0	1	3	2.42	
2:35	12	1	9	0	1	3	2.46	
3:30	11	3	6	1	1	4	2.62	
3:35	11	4	6	0	0	5	2.58	
3:40	11	3	7	0	1	4	2.58	
4:20	14	3	5	0	0	4	2.27	
4:25	14	3	4	0	0	5	2.38	
5:35	17	4	3	0	0	2	1.77	−2.0

jected to stimulation by directive light the photokinetic effects were very marked. The apparent phototactic effects occurred only when these photokinetic effects had become less evident.

Further, the photokinetic effect with Cæcidotea is stronger in comparison with the apparent phototactic effect than it is in the case of Asellus, where the phototactic effect is fairly well marked.

Cæcidotea was subjected to various intensities of horizontal illumination from 5 C.M. (8 c.p. incandescent at 1.3 m. from middle of tank) to 2855 C.M. (772 c.p. 6-glower Nernst lamp at 0.52 m. from middle of tank). No clearly directive responses were obtained to intensities lower than 80 C.M. (19 c.p. at 0.49 m. from middle of tank).

Cæcidotea usually shows a negative response to intensities of 80 C.M. or greater. Table VII shows the results of an experiment with twenty-six Cæcidotea, which had previously been exposed to daylight, when they were subjected to horizontal illumination of an intensity of 2855 C.M. The records were begun five minutes after the animals were transferred to the tank.

This is a fairly typical experiment. The animals having been in the tank only five minutes when the records were begun, the thigmotactic and other influences due to transference to the tank were shown to good advantage. More than an hour elapsed after the beginning of the experiment before any marked indication of a directive response to light appeared, and that response was prob-

ably a chance result since from about 10:30 a.m. (when the first negative response seemed indicated) to 12:00 the mean average position shifted back and forth, at one time apparently indicating a negative response and at another a positive one. From 12:50 to the close of the experiment at 5:35 the apparent negative phototaxis predominated and the other influences became less and less effective. The mean average position during the course of the experiment shifted from 3.77 to 1.77, a movement of 2 in a negative direction.

Fig. 5 represents the results of the experiment recorded in Table VII. The loci of the curve show the mean average positions of the animals during the course of the experiment at intervals which were at first fifteen minutes apart, but later not so close together.

Table VIII shows the results of an experiment similar to the one last discussed. But in this case the animals had been in the tank

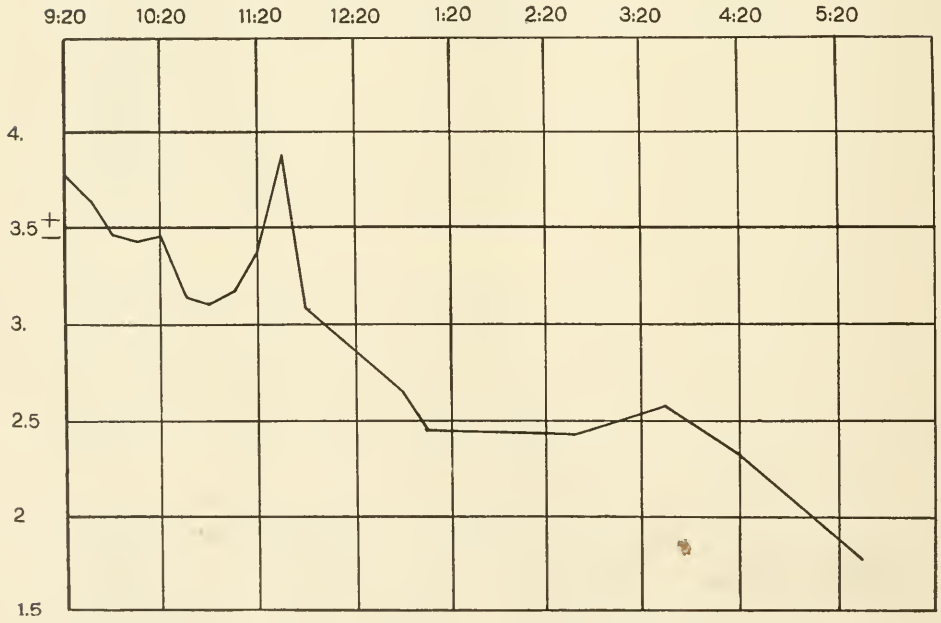

Fig. 5. Cæcidotea stygia (26 individuals); Nov. 7, 1906; previous exposure, diffuse daylight; illumination horizontal, 2855 C. M.

TABLE VIII

CÆCIDOTEA STYGIA (21 individuals)

November 14, 1906

Previous exposure: diffuse daylight

Illumination: horizontal, 2855 C. M.; lamp at Section-1 end

Animals in tank 14 hours before experiment began

TIME OF MAKING RECORDS	+ SECTIONS OF TANK. —						MEAN AVERAGE POSITIONS
	1	2	3	4	5	6	
7:55	5	1	2	7	3	3	3.52
8:00	4	0	1	8	4	4	3.95
8:05	4	0	1	7	5	4	4.0
8:10	3	1	1	7	4	5	4.09
8:15	3	1	3	6	2	6	4.0
8:20	3	2	2	4	5	5	4.0
8:25	2	2	6	6	3	2	3.57
8:30	2	1	5	4	4	5	4.05
8:35	3	1	2	8	6	1	3.76
8:40	1	2	4	6	4	4	4.05
8:45	1	2	4	7	2	5	4.05
8:50	2	3	3	3	4	6	4.05
8:55	2	2	3	5	4	5	4.05
9:03	0	2	5	3	2	9	4.52
9:07	0	1	5	3	2	10	4.71
9:11	2	3	2	4	2	8	4.19
9:15	0	3	3	5	2	7	4.38
9:20	1	3	4	3	2	8	4.24
9:25	1	1	2	6	2	9	4.62
9:30	1	1	3	5	3	8	4.52
9:35	1	1	1	6	2	10	4.76
9:40	1	2	1	6	2	9	4.57
9:45	0	2	1	6	2	10	4.81
9:50	1	1	3	5	2	9	4.57
9:55	1	1	2	4	2	11	4.81
10:00	0	1	1	5	3	11	5.05
10:05	0	1	2	4	1	13	5.09
10:10*	1	1	2	5	1	10	4.7
10:15	2	2	2	3	0	11	4.5
10:20	1	1	3	3	1	12	5.05
10:25	1	3	2	2	0	12	4.65
10:30	2	1	2	1	2	12	4.8
10:35	1	3	0	2	2	12	4.85
10:40	0	1	0	4	0	15	5.4
10:45	0	1	1	2	1	15	5.4

Change in mean average position between the first and the last observation −1.88

*One defective individual removed.

TABLE VIII Continued

Direction of light reversed

TIME OF MAKING RECORDS	− SECTIONS OF TANK.					+	MEAN AVERAGE POSITIONS	
	1	2	3	4	5	6		
10:50	1	2	1	2	1	13	4 95	
10:55	2	2	1	2	4	9	4·55	
11:00	2	2	1	2	2	11	4.65	
11:05	2	2	0	3	4	9	4.6	
11:10	3	1	1	2	1	11	4.58	
11:15	4	1	3	2	1	9	4.1	
11:30	3	3	3	2	0	6	3.65	
11:40	5	4	3	1	0	7	3·4	
11:45	5	4·	2	1	0	8	3·55	
12:50	6	3	4	1	2	4	3.1	
1:05	5	4	3	2	2	4	3.2	
1:10	5	4	3	2	1	5	3·25	
1:15	5	4	4	1	1	5	3.2	
1:20	5	3	4	2	1	5	3·3	
1:25	5	3	5	1	1	5	3·25	
1:30	5	3	5	1	1	5	3·25	
1:40	5	3	4	2	0	6	3·35	
2:35	5	5	4	1	0	5	3·05	
3:30	4	5	4	2	1	4	3·15	
3:45	3	6	5	1	2	3	3.1	
4:30	4	6	4	2	1	3	2.95	
5:35	5	4	6	1	2	2	2.85	Change in mean average position between the first and last observation
								−2.10

14 hours before the experiment with light began, whereas in the former case they had been in the tank only five minutes. Moreover, in the case recorded in Table VIII the light was transferred from one end of the tank to the other during the course of the experiment in order further to test the efficiency of the light as a directive influence.

Since the animals, before this experiment began, had been in the tank over night, the thigmotactic or other disturbing influences due to their transference to the tank was eliminated. The photokinetic effect was therefore easily recognizable. It appeared within a few minutes after the exposure to light began and con-

tinued to be rather conspicuous for about $2\frac{1}{2}$ hours. The records set down in Table VIII were not made at sufficiently frequent intervals to show well the random movements due to this influence. As these movements became less general and less vigorous, the animals were found to be more and more in the negative parts of the tank.

By 10:45, 2 hours and 50 minutes after the experiment started, most of the animals were fairly settled and 18 out of 20 were in the negative half of the tank. This represented a change in mean average position of 1.88 in a negative direction. Possibly further change in this direction would have occurred if the experiment had been continued without modification, but at this time the light was changed to the other end of the tank. Having already been exposed to a high intensity for about 3 hours, the response to this change was not very prompt, and the animals did not shift as near to the new negative end as might have been expected. However, the change in mean average position was very decided in the $6\frac{3}{4}$ hours during which the experiment was continued, being 2.10 in a negative direction.

From these experiments, which are typical of the series, it is evident that Cæcidotea responds negatively to intensities from 80 to 2855 C.M. In general the maximum negative response was reached in approximately 2 to 3 hours, but there was considerable variation in the length of this period depending somewhat upon the intensity of illumination and also upon the length of time the animals had been in the tank before the experiment began. After once responding to horizontal illumination, and becoming fairly well settled in the negative end of the tank, the animals did not move toward the positive end again to any marked extent, i.e., they did not become less negative.

Experiments were made with intensities from less than 1 C.M., (1 c.p. incandescent at 1.3 m. from middle of tank) to 80 C.M. It was shown conclusively that Cæcidotea is never positive in its response to horizontal illumination; but whether or not it is more responsive following retention in darkness or whether it then

responds to lower intensities than when previously in light, was not directly determined. There is, however, convincing evidence that after exposure to strong light for some time Cæcidotea is less responsive to light. This was shown in the experiment detailed in Table VIII, in which at the beginning of the experiment a definite negative response occurred in 2 hours and 50 minutes, three-fourths of the animals under observation having in that time collected and become settled in the extreme negative end of the tank. But when the light was then changed to the other end of the tank, there resulted a less definite response even after a much longer time ($6\frac{3}{4}$ hours) of exposure. The mean average position was changed a great deal, to be sure, but part of that change would have occurred as the result of the normal non-directive movements of the animals. No very pronounced tendency to collect in the extreme negative end appeared in the course of the entire $6\frac{3}{4}$ hours. Hence it seems evident that these animals after exposure to 2855 C.M. for 2 hours and 50 minutes were less responsive to the influence of directive light.

It seems very probable that with Cæcidotea there are not two factors (i.e., phototactic and photokinetic) in the response to horizontal illumination, as there clearly are with Asellus, for with Cæcidotea the whole reaction seemed clearly attributable to photokinesis. Conviction that such was the case led to a partial test of the matter, as the result of which it may be said that no *direct phototactic* reactions were observed with Cæcidotea. The responses were clearly and purely *photokinetic*, the animals starting up in any direction in which they happened to be turned. Definite orientation or direct movements in a negative direction did not occur at all. It was different with Asellus, in which definite orientation and direct responses on the part of individuals very generally occurred.

In order to determine if the difference in luminosity between the two ends of the tank was sufficient to account for the collecting of Cæcidotea in the end farthest from the light under the influence of photokinesis alone, tests were made to determine the actual luminosity in various parts of the tank. The following method was employed. A six-glower Nernst lamp of 772 c.p.

intensity was used. It was placed at the distance of one meter from the nearer end of the outer tank. The photometer was placed close to the opposite end of the outer tank, so that the light from the lamp had to traverse the water in both tanks. The luminosity at the photometer (1.72 meters from the lamp) was measured by using as a standard of comparison a single-glower Nernst lamp whose candle power had been previously determined. In like manner was determined the luminosity at the photometer at the same distance after removal of the tanks. On the basis of these determinations the water used in the tanks in these experiments was found to cut down the light passing through it at the average rate of $\frac{1}{2}$ per cent for each centimeter. By calculation, the luminosity at each part of the tank was then determined. The luminosity at the middle of imaginary section number 1, the section nearest the lamp, was found to be 620 C.M., that at Section 6, 251 C.M., or a little more than two-fifths that at Section 1. When, however, the lamp was placed at a distance of only 20 cm. from the outer tank, the distance at which it often was used in the experiments, the difference in luminosity at the two ends of the tank was still more.

A computation based on the above determination showed that when the six-glower Nernst lamp was used at 20 cm. from the near end of the outer tank the luminosity at the middle of Section 1 was 7863 C.M., whereas at section 6 it was only 1065 C.M. or less than one-seventh as great as at Section 1.

These determinations, while they can be only approximate, show that the difference in luminosity between the two ends of the tank (and the difference would of course be greater the nearer the lamp was to the tank) was very considerable. In order to obtain high intensities of illumination the lamp was, in most of the experiments, used very near to the tank; hence the difference in luminosity between Sections 1 and 6 was usually very great. Since the difference in illumination between the two ends of the tank was so great, there is sufficient basis in that fact for explaining the collection of Cæcidotea in the negative end of the tank as the result of photokinesis alone.

Photokinetic movements were very marked with Cæcidotea

when subjected to an 80 C.M. or a greater intensity, and occurred almost without exception in all such experiments. The response was noticeably more prompt and more vigorous in animals at the end nearer the lamp than in those farther from the source of light. The activity, as stated before, took the form of random movements. There was no evidence of a selection by the individual in the direction of these movements with resulting orientation such as occurs in the random movements discussed by Holmes ('05).

The ultimate positions in which the animals settled were the result of a certain degree of attunement to a definite intensity of light, which rendered them less subject to the photokinetic influence, so that when their random movements carried them into the negative end they were less and less affected by the intensity of light prevailing there and finally became so attuned that the intensity at the negative end of the tank did not affect them in a photokinetic way. Hence the settling at the negative end was the result of photokinesis.

Cæcidotea, although for some time very active, sooner or later became acclimated and came to rest under any intensity of illumination. In the experiment recorded in Table VIII the animals after appearing to respond in a negative phototactic way, became in the course of 2 hours and 50 minutes so acclimated to the existing conditions of illumination that when the light was transferred to the opposite end of the tank, they responded much less definitely than at the beginning of the experiment. Before the experiment began they had been subjected to diffuse daylight; when settled in the negative end of the tank they were acclimated to two-fifths the intensity to which they were subjected after the light was transferred to the opposite end of the tank. These and other similar experiments afford good evidence of acclimatization to light and seem to support the statement that the responses of Cæcidotea to horizontal illumination are due to photokinesis and not to phototaxis.

From the foregoing experiments with Cæcidotea when subjected to horizontal illumination the following conclusions are drawn:

1 Cæcidotea is not very responsive to horizontal illumination.

2 The thigmotactic and other influences due to the transference of the animals to the tank are more potent with Cæcidotea than with Asellus, and the effect continues longer.

3 Cæcidotea does not respond to intensities of illumination less than about 80 C.M.

4 Cæcidotea responds negatively to intensities from 80 C.M. to 2855 C.M.

5 This response is not direct, i. e., it is not a direct phototactic response.

6 Light produces with Cæcidotea a very decided photokinetic effect, which continues for some time. As this effect decreases, the animals gradually settle at or near the negative end of the tank, i. e., they gradually become acclimated to the lower intensity of illumination, and when acclimated to the conditions of illumination at the negative end of the tank they accumulate there.

7 With horizontal illumination both Asellus and Cæcidotea respond in a negative way to moderate intensities as well as to all stronger intensities; but below certain ranges of intensity both are indifferent. Cæcidotea is indifferent to light intensities below 80 C.M., Asellus to intensities below 2.5 C.M. Hence Cæcidotea is indifferent to a considerable range of intensities (2.5 C.M. to near 80 C.M.) to which Asellus is more or less responsive.

8 However, after having been in the dark for several hours, Asellus is positive to intensities of light between 2.5 C.M. and about 80 C.M.; and sometimes it is positive for a short time to an intensity of 2855 C.M., but in that case it very quickly becomes negative. After considerable exposure to any intensity to which it responds at all, Asellus is positive.

9 Although Asellus is not very responsive to horizontal illumination, it is decidedly more responsive than Cæcidotea to those intensities to which it responds at all. This difference appears in the directness and the relative promptness of the response given by Asellus as compared with the irregular and tardy response of Cæcidotea.

10 This difference in the reactions of Asellus and Cæcidotea

to light is sufficient to account both for the occurrence of Cæcidotea in caves and subterranean waters in general and for the virtual non-occurrence of Asellus in such situations; for the negative response of Cæcidotea to light would aid in directing it into caves and keeping it there; but Asellus after being in darkness becomes positive, and therefore would move toward the light, i.e., out of a cave, in case it had by chance made its way into one.

II. VERTICAL ILLUMINATION

1. Methods and Apparatus

The apparatus used in the experiments with vertical illumination is shown in vertical section in Fig. 6 (p. 281), a dark and a light field were secured by using the same two tanks as in horizontal illumination and causing one-half of the inner tank to be illuminated by vertical rays while the other half was kept as dark as possible. A rectangular lamp-container (*LC*), similar to the one used in the experiments with horizontal illumination, was suspended in a vertical position by means of two stout cords passed through pulleys so that it could be raised or lowered as desired. A broad V–shaped piece of blackened sheet iron (*C*) was placed as a roof over the top of the lamp container in such a way as to prevent the light from escaping above into the room and at the same time to afford a ready means for the escape of the heated air from around the lamp. Within the lamp-container at one side was fitted an adjustable partition (*P*) made of two thicknesses of pasteboard. This partition was lengthened at the lower end by means of a heavy card-board fastened between the two thicknesses of pasteboard in such a way that it could be moved freely in the plane of the partition. The lower edge of this partition was made to bisect the inner tank, fitting into it closely and extending down to the surface of the water when the tank was filled to a depth of 3 cm. The partition was practically light proof; but in order the more effectually to shut out all light from the dark end of the tank, that end was carefully covered over with a black cloth supported by a large piece of blackened pasteboard (*S*). Black cloth was

tightly fastened around the lower end of the lamp-container and closely drawn around the illuminated end of the tank as well as the edges of the partition, so as to form a light-tight hood between

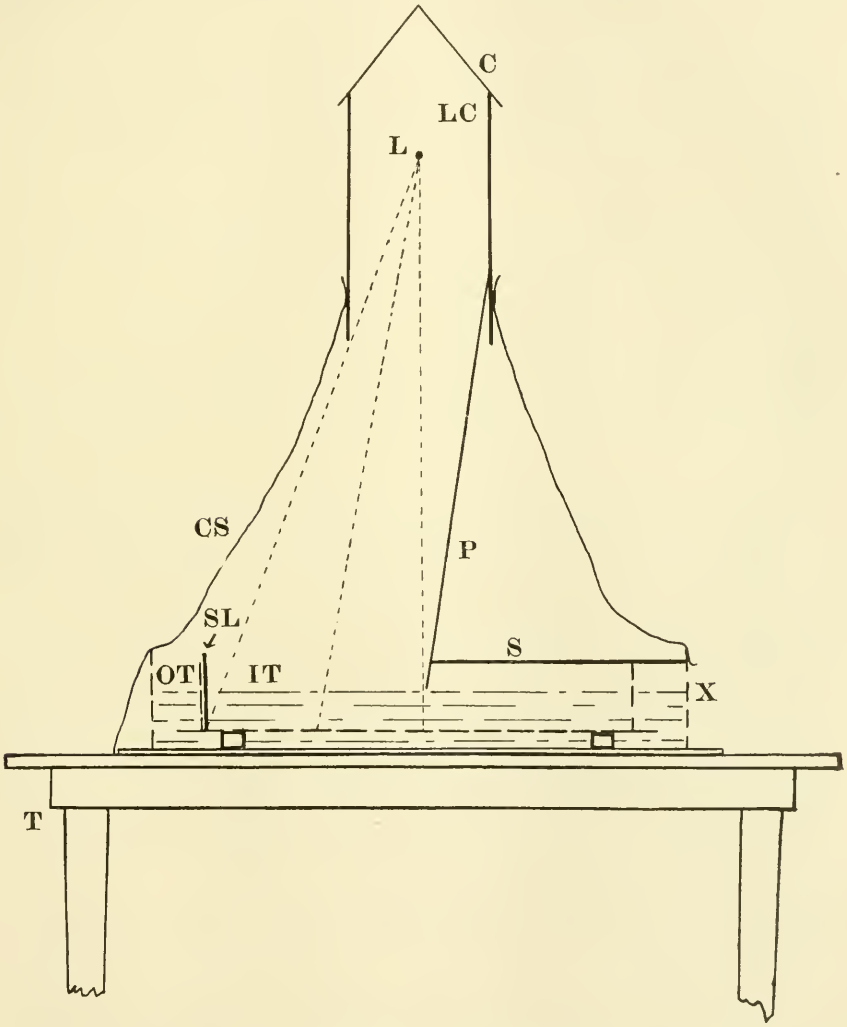

Fig. 6 Diagram showing in vertical section the apparatus used in experiments with vertical illumination. *C*, sheet-iron roof of lamp container; *CS*, cloth screen; *IT*, inner tank; *LC*, lamp container; *OT*, outer tank; *P*, partition; *S*, horizontal screen; *SL*, vertical slate; *T*, table; *X*, place of observation.

the lamp-container and the light end of the tank. The only light which could reach the dark region came through the water from the illuminated half of the tank. A 6-glower Nernst lamp suspended within the lamp-container was the one most used. The partition was set at such an angle within the lamp-container that the middle of its edge was immediately below the Nernst lamp This secured rays of light at right angles to the long axis of the tank at the plane of division between the dark and the light regions.

In these experiments a sharp plane of separation between a strongly illuminated and an entirely dark region was desired. To the eye this plane seemed very distinct, though the dark region was somewhat illuminated, perhaps to one-fiftieth the intensity of the light region, due to the diffusion of light through the water and also to reflection from the ground glass bottom of the inner tank. The sides and end of the illuminated half of the tank were non-reflecting, since they were lined with sheets of slate painted dead black.

Usually the observations were made from a position (*X*) near the dark end of the tanks, the eye being placed slightly below the level of the water in the tank. By looking through the glass ends of the two tanks and the water within, one was able to observe the whole surface of the inner tank and the animals upon it without changing his position or disturbing the light hood. High intensities of illumination were generally used and little difficulty was experienced in counting the animals in either the light or dark regions. If the illumination was not sufficient to enable one to see readily the animals in the darkened region, their outlines could be observed by bringing the eye into such a position that the animals would appear silhouetted against the relatively intensely illuminated space beyond. This method of observation was seldom necessary, however.

The 6-glower Nernst lamp was used at a distance of about 30 cm. from the floor of the tank. It produced considerable heat at the surface of the water, but the heat thus produced was not apparent at the bottom of the tank, for it was not enough to affect a thermometer bulb placed there. According to Melloni (cf. Mast

'06, p. 387, footnote) a layer of distilled water 9.21 mm. thick transmits only 11 per cent of the total incident radiation. Tap water transmits only a very little more. Hence the amount of heat reaching the bottom of the above mentioned tank would be less than 0.2 per cent of the total incident radiation.[2]

To guard against a rise in temperature of the water in the experimental (inner) tank, the water of the outer tank was constantly renewed by siphons, one drawing off the water at one end while the other replenished the tank at the other end. This arrangement proved effective.

The intensity of illumination at the bottom of the tank, allowing ½ per cent reduction in actual luminosity for each centimeter of water through which the light passed (see page 277), was 6983 C.M. (772 c.p., 6-glower Nernst lamp at 30 cm. from surface of water, which was 3 cm. deep). The luminosity in the dark region of the tank, while not determined, was apparently near, though below, the threshold of stimulation for directive response in Asellus, and certainly much below that in Cæcidotea.

Sometimes the experiment was started by placing the animals in the illuminated part of the tank and observing their reactions at once. At other times the animals were placed in the tank and allowed sufficient time to become thoroughly settled before they were exposed to the light. Separate treatment of the experiments on the basis of this difference is unnecessary, for the disadvantage of the random movements of the animals in the former case was balanced by the disadvantage of the apathy of the animals in the latter case. In both the ultimate responses were the same.

Numbers of individuals were experimented with at the same time, as in the experiments with horizontal illumination.

[2] If the first 9.21 mm. of water transmits 11% of the incident radiation, the second 9.21 mm. would transmit 11 per cent of *its* incident radiation, i. e., 11 per cent of 11 per cent or 1.21 per cent of the incident radiation at the surface of the water.

The third 9.21 mm. of the water would transmit 11 per cent of *its* incident radiation, or 0.133 per cent of the incident radiation at the surface of water.

The remaining 2.37 mm. of water would still further reduce the amount of heat reaching the bottom of the tank.

2. *Asellus*

As in all other cases, Asellus and Cæcidotea were experimented with in succession and under the same conditions. The reactions of Asellus will be described first.

With vertical illumination of 6983 C.M.[3] intensity the photokinetic effect upon Asellus was very marked. If the animals had only recently been placed in the tank, the random movements brought about by thigmotaxis or other influences due to transference to the tank were likewise very marked, but because of photokinesis the animals in the illuminated region were more active than those in the dark region. When the influence of the transference to the tank ceased, the difference in activity between the animals in the illuminated region and those in the dark region was increased. When given time to become acclimated to the tank before the experiment was begun, many of the animals began to move about within a half minute or less. With Asellus the characteristic intermittent movements continued in most cases as long as the animals were in the light region, but the activity was always less when they were in the dark region, so that there was a decided photokinetic effect.

Concerning the usual movements of Asellus, it may be said that normally the animal moves by short stretches or "runs," between which it pauses for a time. When more active, its runs are longer and its pauses shorter. It normally comes to a stop by a gradual slowing of its movements, as it might appear to do if the stop were due to a loss of momentum. When stimulated in any manner just after the beginning of one of these runs, or near the end of it, the animal often stops almost instantly. Sometimes, however, the movement is immediately accelerated by the stimulus, for the run is more rapid and longer than it would otherwise have been. If the stimulus is applied during the middle of a run, it seems less likely to be effective. When an Asellus has been stop-

[3] This intensity was produced by the use of a 772-c. p., 6-glower Nernst lamp at 30 cm. from the surface of the water, which was 3 cm. deep. In calculating the intensity of the light at the bottom of the dish, where the animals were, ½ per cent was deducted for each cm. of water through which the light passed.

ped by some stimulus, it often remains perfectly quiet for a time. But if the stimulation be kept up, the animal usually waves its antennæ about in a characteristic manner, lifts its head slightly, turns the anterior end of the body to one side or the other a little, and perhaps turns the entire body more or less and begins to move again. All of these movements may occur in succession, or any one may occur without the others, or only the first part of the series may be gone through with; but the series occurs often enough to be characteristic, particularly with stimuli that are non-directive. This strongly suggests a motor reaction in the sense in which Jennings uses the term. It is not that stereotyped type of reaction, however which characteriszes the typical motor reaction.

With these preliminary statements regarding the actions of Asellus, we are in position to consider the actions of the species at the plane of division between the dark and the light regions.

If headed toward the illuminated region, the animals sometimes stop abruptly when partly across the plane or immediately after crossing it. This stopping occurred often enough to indicate that it was due to the sudden action of the light on the animal. If the animal in one of its runs, reached the plane before it was well under way, or when it seemed near the end of such an excursion, this abrupt stop was more likely to occur than if the animal crossed the plane while well under way, since in the latter case any stimulus, as has been stated, is likely to be less effective than when the animal is moving more slowly. Sometimes stopping near the line was apparently due to causes other than that of suddenly coming into the light, e. g., the animal may have reached the end of its run. But in other cases the stopping was so abrupt and the reaction so characteristic of Asellus when stimulated, that without question the reaction was due to the influence of the light.

If the animal stopped at the plane, or so little beyond it that the characteristic movements following stimulation brought its head back partly or entirely over the plane, it almost invariably turned into the dark region at once or followed along the plane a short distance and then entered the dark. Animals which on entering the light region met the plane at a very oblique angle

seldom failed to turn back into the dark region, unless they crossed the plane in the middle of a run. It must not be thought, however, from the above statement that an immediate turning back into the dark region was the rule. For, in the first place, the majority of the individuals crossed the plane without stopping at all, and secondly, not more than one-fourth of those which appeared to be stopped by the sudden illumination, turned directly back. The sequence and character of the reactions at the plane were by no means invariable. The animals, even when apparently made to stop by the influence of the light, sometimes merely waved the antennæ somewhat, or lifted the head a little and moved on in the same direction as before. Animals which the sudden effect of the light did not cause to stop when going into the illuminated area sometimes showed an immediate acceleration in movement. If such acceleration in movement did not occur at once it appeared very soon. The same statements may be made with reference to those individuals which stopped at the plane but did not find their way back into the dark region at once; after the first pause they showed quickened movements either at once or very soon thereafter. Hence, in any case, though the animal might pause on first entering the illuminated region, photokinetic effects very soon appeared.

Of more general occurence than the stopping or turning back of the animals upon entering the illuminated region was the stopping of the animal just within the dark region when entering it from the light. It frequently happened that soon after starting an experiment in which 25 or 30 Asellus were used, from four to eight individuals at a time would be seen just within the dark region, where they had stopped as soon as their impetus allowed after passing beyond the reach of the light stimulus.

No attempt was made to determine the reaction time at the plane, but the distances beyond the plane of the positions at which the animals stopped were apparently determined by the individuals's reaction time and its momentum when it reached the plane.

Occasionally the animals, after starting across the plane into the dark region, would turn back into the illuminated region, but this was exceptional.

A few of the records of observations on the actions of individual Asellus at the plane of division between th e light and the dark areas are here transcribed to illustrate the details upon which are based the general statements which precede.

April 28, 1906. *11:10.* One Asellus climbed up side of tank at the plane. Came from dark and returned to dark.

11:15. An individual came from dark headed into light and immediately turned about.

11:25. A large ♂ moved back and forth across the plane several times and for eight minutes did not get far from the plane. It finally went into dark.

2:15. One individual came to the plane from light, paused and then crawled up side just on the plane remaining on plane for approximately a minute, then went to dark.

2:15. At start it was noted that after the Asellus crossed the plane into dark they often paused much longer than usual. At one time five were noted just within the dark, all having heads toward dark.

2:32. One individual headed into light but soon turned back, then moved along plane for sometime, moving partly into light, and after about 1½ minutes turned away into dark.

2:32. Another went into light and immediately hurried off and scarcely stopped until it had made a circuit of tank and gotten into dark, where it stopped for some time and then moved away very deliberately.

2:37. One started into the light end but stopped as soon as it reached the plane, and turned back in two or three seconds.

The above were notes made hastily the first time Asellus was observed under these conditions, and are given here, first, because they show characteristic reactions of the animals; and, secondly, because they were made before I had formed any very definite conception of the movements and actions of the animal under these conditions of experimentation, and when I was entirely unprejudiced toward any explanation of the animal's actions at the bounding plane.

The eyes of Asellus, as might be expected, played a conspicuous part in the reactions at the bounding plane. Whether the animal was headed from the illuminated region toward the dark region, or vice versa, the effects appeared as soon as the eyes were across

the plane. If the animal, upon turning back after it had crossed over into the illuminated region, got into such a position that one eye was in the dark region, its immediate return to the dark space was almost certain. Meeting the plane at a sharp angle brought one eye into the light, while the other was still in the dark; hence the turning back into the dark that almost invariably occurred in such cases. When an Asellus followed along the bounding plane for a short distance before turning into either area, the head was the part of the body which "found" the plane and directed the animal in following it. Whatever the direction of the animal's movements, they were never modified *until the eye reached the bounding plane.* This was taken to indicate that the animal when approaching the illuminated region was not capable of distinguishing that region until the eyes were very near the plane or were actually illuminated by the strong light; likewise that when moving in the light toward the dark region it was equally incapable of detecting that region until the eyes were quite near the plane or actully carried beyond the reach of the light. The actions of the animal in following along the plane and in finding its way back into the dark region are clearly due to the effects of unsymmetrical stimulation of the two eyes.

The general movements of the animals as a whole and the ultimate positions taken by Asellus under such conditions will next be considered.

In Table IX are given the details of an experiment with vertical illumination by means of the apparatus previously (p. 280) described. In this case 24 individuals (Asellus) were transferred to the tank and left for one hour in diffuse daylight. The room was then darkened and the artificial light (6983 C.M.) turned on. The observations given above (p. 287) were made during this experiment. In the first column, at the left, are given the epochs at which observations were made; in the two other columns are given the numbers of individuals found in the illuminated region and in the dark region respectively at these epochs.

At the beginning of this experiment the number of individuals in the illuminated end was very nearly equal to that in the dark end. But the photokinetic effect appeared very promptly, and

TABLE IX

Asellus communis (24 individuals)

April 28, 1906

Previous exposure: diffuse daylight

Animals in tank for 1 hour.

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION (6983 c. m.)	NUMBER IN DARK REGION
10:20	11	13
10:21	10	14
10:22	8	16
10:23	4	20
10:24	6	18
10:25	9	15
10:26	11	13
10:27	15	9
10:28	12	12
10:29	12	12
10:30	12	12
10:31	7	17
10:32	5	19
10:33	2	22
10:34	3	21
10:35	4	20
10:36	4	20
10:37	6	18
10:38	9	15
10:39	10	14
10:40	11	13
10:41	9	15
10:42	7	17
10:44	6	18
10:45	6	18
10:46	6	18
10:47	9	15
10:48	7	17
10:49	9	15
10:50	9	15
10:51	10	14
10:52	12	12
10:53	9	15
10:54	8	16
10:55	7	17
10:56	10	14
10:57	9	15
10:59	5	19

TABLE IX Continued

TIME OF MAKING RECORD	NUMBER IN ILLUMINATED REGION (6983 c. m.)	NUMBER IN DARK REGION
11:00	6	18
11:01	6	18
11:02	4	20
11:03	2	22
11:04	1	23
11:05	1	23
11:06	4	20
11:07	5	19
11:08	4	20
11:09	3	21
11:10	1	23
11:11	3	21
11:12	4	20
11:13	4	20
11:14	3	21
11:15	4	20
11:16	3	21
11:17	4	20
11:19	5	19
11:20	9	15
11:21	8	16
11:22	9	15
11:23	9	15
11:24	9	15
11:25	7	17
11:26	6	18
11:27	5	19
11:28	3	21
11:29	2	22
11:30	3	21
11:31	3	21
11:33	2	22
11:34	1	23
11-35	1	23
11:36	1	23
11:37	2	22
11:40	2	22
12:10	1	23
12:35	2	22
Average for the whole time.	6−	18+
Average per cent for whole time.	24.9%	75.1%

the number in the illuminated end rapidly decreased. However, the photokinetic effect usually did not cease at once when the animals entered the dark region, hence many of them on reaching the end of the tank, turned back and often wandered into the illuminated region again. Since in this experiment the animals had been in the tank but an hour, the thigmotactic or other influence due to the transference of the animals to the tank was still effective. These influences caused the animals to move about so vigorously that they kept entering the illuminated region rather freely for over an hour. However, since the activity was less in the dark end, that factor alone served to keep the number in the dark region in excess of that in the illuminated region. When the thigmotactic and photokinetic influence became less strong the number in the illuminated region became quite small and remained so. The observations of Table IX will serve to illustrate the nature of the experiments of this series, which are summarized in Table X. In all these the Asellus were confined in the tank, one half of which was exposed to vertical illumination of 6983 C.M., while the remaining half was very faintly illuminated.

This series of experiments shows Asellus to be extremely responsive to vertical illumination of 6983 C.M. intensity. In the end virtually all of the animals, remained in the dark region entirely out of range of the strong light.

By way of general summary of this series of experiments with Asellus the following conclusions are drawn:

1 The photokinetic effect in the illuminated end of the tank is very marked.

2 Photokinesis causes some of the animals to start up suddenly soon after they are exposed to the light, and a generally increased activity soon results.

3 When headed toward the illuminated space the sudden illumination of the animal upon crossing the plane between the dark and the illuminated region sometimes causes the animal to stop abruptly.

4 When the animal is made to stop by this sudden illumination, it often reacts in a characteristic way.

TABLE X

ASELLUS COMMUNIS

Intensity of illumination, 6983 C. M.

DATE	LENGTH OF TIME COVERED BY EXPERIMENT	NUMBER OF ANIMALS USED	PREVIOUS EXPOSURE	TIME IN TANK BEFORE EXPERIMENT BEGAN	PER CENT IN ILLUMINATED REGION AT START	PER CENT IN ILLUMINATED REGION IN GIVEN TIME AFTER EXPERIMENT BEGAN											BEGINNING OF COLLECTING IN THE DARK	MAXIMUM RESPONSE VIRTUALLY PRODUCED	AVERAGE PER CENT IN ILLUMINATED REGION*
						5 min.	10 min.	15 min.	½ hr.	¾ hr.	1 hr.	1¼ hr.	1½ hr.	1¾ hr.	2 hr.	2¼ hr.	2½ hr.		
	hours			hours														min.	min.	
April 28, 1906.....	2¼	24	Daylight	1	46	38	50	8	38	4	38	4	6	4	6	8		2	75	25
April 28, 1906.....	¾	24	"	4	58	25	25	28	13	12								1	30	16
Oct. 6, 1906	1½	32	"	0	56	50	47	50	38	34	38	31	23					15	30	40.6
Oct. 9, 1906	2½	40	"	0	50	43	43	30	40	45	50	25	35	18	30	28	23	5	60	32.8
Oct. 9, 1906.....	2¼	40	"	4	35	15	18	20	15	20	10	20	13	8	8	13		1	36	12.8
Oct. 11, 1906	1¼	43	"	0	100	60	33	42	33	42	47	23						2	60	37
Oct. 11, 1096.....	1½	43	Darkness	2¾	35	12	12	12	7	23	14	5	5					1	30	16
Oct. 12, 1906.....	1½	43	Daylight	22	74	60	37	28	9	5	5	2						7	30	14.1
Averages for entire set of experiments																		4.3	44	24.3—

*The average percents given in the last column are taken from the data of the entire experiment, not merely from these abstracted data.

5 This characteristic reaction sometimes brings the animal's eyes back to the plane, in which case the animal seldom fails to return into the dark region.

6 Animals which meet the plane at a sharp angle usually enter the dark regions whatever may be the direction in which they are headed.

7 Individuals which, in going into the illuminated region, pass the plane without being stopped by the sudden influence of the light, sometimes show increased activity almost instantly. At other times the increase comes less quickly.

8 Individuals entering the dark region from the illuminated one *very generally* stop on crossing the plane separating the two, but in cases where the individual does not stop a decrease in activity occurs.

9 The eyes are the effective light receptive organs of this animal, as is shown by the actions of the animal at the plane between the dark and light regions.

10 Unsymmetrical stimulation of the two eyes governs the animal's movements at the plane.

11 The photokinetic effect keeps the animals moving for some time. It causes them to recoil from the dark end of the tank and then to continue to go back into the illuminated area.

12 More than half of the animals collect within the dark region on the average within 4.3 minutes after the experiment starts. Virtually all the animals ultimately settle down and remain in the dark region of the tank, this occuring on the average 44 minutes after the experiment starts.

3. Cœcidotea

The experiments upon Cœcidotea with vertical illumination were made under exactly the same conditions as were those upon Asellus. However, two preliminary experiments were made upon Cœcidotea with intensities lower than 6983 C.M. In one of these experiments (the bottom of) one end of the tank was illuminated with an intensity of 97 C. M. (60 c.p. incandescent at 75 cm. from surface of water); in the other the intensity was 172 C.M. (49 c.p.

Nernst at 50 cm. from surface of water). In the first case 12 animals were used and the experiment was continued for 3 hours. In the second 24 animals were used for $2\frac{1}{2}$ hours. Little or no photokinetic effect appeared in either case, and the animals did not exhibit a tendency to remain in the dark region rather than in the light one. When two days later, the same animals were exposed to 6983 C.M. intensity, very definite reactions were obtained. It is therefore safe to conclude that Cæcidotea is little responsive to vertical illumination of an intensity of 172 C.M. or less.

As with Asellus, vertical illumination of 6983 C.M. intensity produced a decided photokinetic effect upon Cæcidotea.

Random movements due to recent transference to the tank likewise appeared in the experiments with Cæcidotea. But such movements are of only passing interest here, for they merely resulted in a delay in the ultimate settling of the animals. During the period of these random movements the activity was greater in the illuminated than in the dark area. This difference in activity in the two areas became more evident as time passed, the influence due to recent transference to the tank presumably becoming less strong.

The photokinetic effect was less marked and less prompt in its appearance with Cæcidotea than with Asellus, but there was no mistaking its existence. However, in the case of Cæcidotea, when it had once become settled in the tank, the response to this influence was quick compared with the usual tardy response to light. With many individuals the response came in the course of a minute or two, and the majority of the individuals were stimulated to begin moving within ten minutes.

From general preliminary observations it seemed that Cæcidotea sometimes reacted in a direct and definite manner to the change in illumination at the plane of division between the illuminated and the dark regions. In a general way these reactions were like those of Asellus under like conditions, but they occurred much less often, so that it was hard to decide whether there really were definite reactions, and whether the observed actions at the bounding plane were not due to other causes than the change in illumination. To test these supposed reactions of Cæcidotea an arbitrary

TABLE XI

Cæcidotea stygia (18 individuals)
October 5, 1906
Previous exposure: diffuse daylight in tank, for 1½ hours.

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION
10:36	16	2
10:37	17	1
10:38	16	2
10:39	13	5
10:40	13	5
10:41	13	5
10:42	13	5
10:43	11	7
10:44	11	7
10:45	10	8
10:46	10	8
10:47	11	7
10:48	10	8
10:49	11	7
10:50	11	7
10:51	11	7
10:52	9	9
10:53	9	9
10:54	8	10
10:55	7	11
10:56	8	10
10:57	7	11
10:58	7	11
10:59	9	9
11:00	9	9
11:01	8	10
11:02	8	10
11:03	10	8
11:04	8	10
11:05	7	11
11:10	6	12
11:11	7	11
11:12	9	9
11:13	10	8
11:14	10	8
11:15	9	9

TABLE XI Continued

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION
11:16	7	11
11:17	7	11
11:18	7	11
11:19	6	12
11:20	8	10
11:21	9	9
11:22	8	10
11:23	7	10
11:24	8	10
11:25	7	11
11:26	7	11
11:27	5	13
11:28	5	13
11:29	5	13
11:30	5	13
11:31	7	11
11:32	7	11
11:33	8	10
11:34	8	10
11:35	8	10
11:36	8	10
11:37	6	12
11:38	5	13
11:39	6	12
11:40	7	11
11:41	7	11
11:42	6	12
11:43	5	13
11:44	4	14
11:45	5	13
11:46	4	14
11:47	5	13
11:48	5	13
11:49	5	13
11:50	6	12
11:51	6	12
11:52	6	12
11:53	7	11
11:54	7	11
11:55	7	11
12:00	6	12

TABLE XI Continued

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION
12:05	5	13
1:09	3	15
1:10	3	15
1:11	3	15
1:12	2	16
1:13	3	15
1:14	3	15
1:15	3	15
1:16	3	15
1:17	3	15
1:18	3	15
1:19	3	15

TABLE XI Continued

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION
1:20	3	15
1:21	3	15
1:36	3	15
Average for whole time...	7.3—, or 40.5%	10.7+, or 59.5%
Average after one hour	4.6—, or 25.5%	13.4, or 74.5 %
Average after 2½ hours.....	2.9—, or 16.3%	15.1+, or 83.7%

plane was established in the middle of the illuminated region for comparison with the boundary between the light and dark regions. The hood around the illuminated part of the tank was then opened somewhat to permit observations from above, and the actions of the animals when they passed through the arbitrary plane and likewise when they passed the boundary between the dark and illuminated regions were carefully watched and compared.

It was found, after observing Cæcidotea passing through these planes repeatedly, that the reactions at the bounding plane between light and dark were not very marked. However, out of a total of 2126 observations at the two planes, 50 cases were noted in which the animal after starting across the bounding plane into the illuminated region turned about and remained within the dark region whereas only 30 cases of similar sharp turning about of animals moving away from the dark half of the tank occurred at the *arbitrary* plane. Conversely, only 16 cases were noted in which the animals upon entering the dark region from the illuminated one turned back sharply and remained within the illuminated region, whereas 23 cases were noted in which a similar turning about of animals moving toward the dark region occurred at the *arbitrary* plane. There were 208 cases in which the animal on entering the dark from the illuminated regions stopped when crossing the

boundary plane; only 172 such pauses occurred at the arbitrary plane. These results, while not very decided, still indicate that Cæcidotea is sometimes affected by the abrupt change in the intensity of illumination at the plane of separation between the light and dark regions.

The general movements of Cæcidotea, when experimented on in numbers, under the conditions already described (one half of the tank being dark, the other half being under vertical illumination of 6983 C.M.) were fairly definite and no lengthy series of experiments was necessary to demonstrate them. In addition to the general photokinetic effect, which incited the animals to movement and kept those in the illuminated region more active than those in the dark one, there existed a very pronounced tendency for the animals to congregate in the dark region.

Table XI gives the results of a characteristic experiment of the series. In this experiment 18 Cæcidotea were placed in the tank already described and under the same conditions of vertical illumination (6983 C.M. intensity) as were employed with Asellus (p. 288). These animals had been in the tank in diffuse daylight for 1½ hours before the records began, but just before the light was turned on they were all driven into the region that was about to be illuminated. Naturally, if all the conditions were the same in both halves of the tank, one would expect the animals to distribute themselves equally in the two. But when, the other conditions remaining the same, the light conditions are different in the two halves, any marked difference in distribution is clearly attributable to reaction to light.

In this experiment, starting with practically all the animals in the illuminated region, an equality of distribution had become established in the course of 16 minutes. But this equality did not persist; after a few fluctuations on either side of equality during the next period of about thirty minutes, the number in the dark region became permanently greater than that in the illuminated part, and although there were some fluctuations, the tendency was toward a constant increase of numbers in the dark region, which finally reached a maximum in about two and a half hours. Although this result was accomplished rather slowly, it

TABLE XII

Cæcidotea stygia

Intensity of illumination, 6983 C. M. Animals exposed to daylight before the beginning of experiments

DATE	DURATION OF EXPERIMENT	NUMBER OF ANIMALS USED	TIME IN TANK BEFORE EXPERIMENT BEGAN	PER CENT IN ILLUMINATED REGION AT START	PER CENT IN ILLUMINATED REGION IN GIVEN TIME AFTER EXPERIMENT BEGAN											BEGINNING OF COLLECTING IN THE DARK	MAXIMUM RESPONSE VIRTUALLY PRODUCED	PER CENT IN LIGHT AT CLOSE	AVERAGE PER CENT IN ILLUMINATED REGION*
					5 min.	10 min.	15 min.	30 min.	45 min.	1 hr.	1¼ hr.	1½ hr.	1¾ hr.	2 hr.	2½ hr.	3 hr.				
	hour.																min.	hr.		
April 14, 1906.....	1¾	24	0	75	—	—	58	29	29	33	—	—	25				30	1¾	25	33.6
April 14, 1906.....	1	21	5½ hrs.	100	57	43	43	33	—	19							12	1 1/30	19	41
April 18, 1906	1	24	0	61	—	52	34	33	25	20							12	1	20	31
April 26, 1906.....	2½	11	11 min.	63	56	63	31	27	27	28	20	11	18	36	30		14	1½	30	33
Oct. 5, 1906	3	18	1½ hrs.	89	73	56	61	39	44	44	33	28	—	—	17	17	18	2½	17	39.2
Oct. 6, 1906.....	2½	15	0	87	80	60	50	42	54	42	25	40	40	38	17		18	1¼	17	37.3
Oct. 10, 1906.....	3	20	0	90	52	47	63	42	28	55	21	17	37	42	22	22	30	1½	22	34.3
Oct. 13, 1906	2	23	0	57	22	35	17	13	22	4	13	—	—	4			2	½	4	13
Averages for entire set of experiments																	17	1 h. 18 m	19.2	32.8

*The averages in the last column are taken from the data of the entire experiment, not merely from these abstracted data

was sufficiently obvious and definite, as it also was in all other similiar experiments. For the whole time of the experiment the average number of animals in the illuminated area was 40.5 per cent of the entire number of individuals in the tank, but for the last half hour only 16 per cent stayed in the illuminated portion.

Table XII gives in condensed form the results of the experiments of this series.

From this table it may be seen that the average length of time from the beginning of the experiment until the majority of the animals began to collect in the dark region of the tank was 17 minutes, and the average time until the maximum response had been virtually reached was 1 hour and 18 minutes. The average per cent remaining in the illuminated region for the whole time of the experiments was 33 –, and the average per cent remaining in the illuminated region at the close of the experiment was 19.2. Hence it seems clear enough that under these conditions of illumination the Cæcidotea tend to collect in the dark end of the tank and that once collected and settled, they remain there.

If it be granted that Cæcidotea sometimes reacts to the abrupt change in illumination at the plane of division between the dark and the illuminated regions, then the reactions are due to the effects of unequal stimulation upon the different parts of the animal's body, and to a tendency in the animal to react in a characteristic manner when strongly stimulated. This characteristic reaction in Cæcidotea consists in a more or less abrupt turning about and starting off in a new direction. This reaction may aid the animal in getting partly within the dark region when once entirely across the plane and in the illuminated region. This occurs so rarely, however, as to be practically negligible.

Those general reactions to the light which were manifest in an increased activity in the illuminated region were of course photokinetic, and the collecting of the animals within the dark region was likewise due to photokinesis. The animals were from the first more active in the illuminated than in the dark region. This alone would soon cause individuals to collect in the dark region, where the activity was less. When the photokenetic effect ceased to be sufficient to cause the animals to recoil from the end of

the tank in the dark region so as to reënter the illuminated region, then the animals remained in the dark region.

The foregoing experiments indicate:

1 That under the conditions described, Cæcidotea was little responsive, either in increased activity or in collecting within the dark area, when exposed to intensities of 97 C.M. and 72 C.M.; but

2 That with an intensity of 6983 C.M., a very decided photokinetic effect was produced;

3 That this photokinetic influence caused most of the animals to become active in from 30 seconds to about ten minutes after they were exposed to the light;

4 That thus the activity was greater in the illuminated region; less pronounced in the dark region;

5 That sometimes Cæcidotea, like Asellus, apparently reacted at once to the sudden transition in illumination between the dark and the illuminated regions;

6 That this reaction sometimes appeared as a sudden turning back into the dark region after the stopping at the plane between the dark and the illuminated regions, when headed towards the illuminated part.

7 The influence of the change in illumination was also shown by the fact that Cæcidotea when headed toward the dark region, did not often turn back into the illuminated region after stopping at the bounding plane; whereas, when headed in the opposite direction such turnings into the dark region were not rare.

8 In addition to the general photokinetic effects upon Cæcidotea the vertical illumination of 6983 C.M. intensity caused the animals ultimately to collect and remain within the dark region.

9 The majority of the animals began to appear in the dark portion of the tank within 17 minutes after the beginning of the experiment.

10 Practically the maximum number was collected in the dark region within about $1\frac{1}{4}$ hours, and when once settled there they remained in that region.

11 Certain reactions of Cæcidotea at the plane of division between the dark and the illuminated regions are apparently due to

the effects of unequal illumination upon different parts of the animal's body.

12 Cæcidotea sometimes reacts in a characteristic manner to the sudden influence of the light, and this occasionally assists in directing the individual into the dark region.

13 The general reactions, consisting in increased activity within the light region and an ultimate assembling in the dark region, were due to photokinesis. The assembling and remaining within the dark region occurred when photokinesis was not strong enough to cause the animals to recoil from the dark end of the tank so as to reënter the illuminated region.

Nagel ('94) has called the reactions of animals to sudden illumination "photoskioptic" and to sudden shading "skioptic." Applying Nagel's terms here, it may be said that both Asellus and Cæcidotea are both photoskioptic and skioptic, since both species (or certainly Asellus) sometimes immediately respond to the sudden change in illumination encountered in passing from a dark region to an illuminated one, and also on passing from an illuminated to a dark region. Yerkes ('03, p. 306) states that with the jelly fish Gonionemus murbachii, an "increase in light intensity uniformly causes a motor reaction in quiescent individuals, and the inhibition of movement in active individuals. Decrease in light intensity usually causes the inhibition of movement in active individuals, but rarely does it act as a stimulus to activity in case of resting animals."

With Asellus and Cæcidotea the photoskioptic and skioptic responses are more variable than in Gonionemus. Both species of crustacea when active sometimes respond to a sudden increase in the intensity of illumination by an inhibition of movement, at other times by an abrupt acceleration of movement; when quiescent, Asellus often responds to increased intensity of illumination by moving at once. If in motion, Asellus very often responds to a sudden decrease in the illumination by coming to an abrupt stop.

These experiments with vertical illumination have shown that both species respond in a photokinetic way and ultimately collect in a dark region. Asellus responds more quickly and more gener-

ally than Cæcidotea, smaller percents of Asellus than of Cæcidotea entering and remaining within the light region. Asellus very generally reacts at once to the sudden change in illumination at the plane of separation between the illuminated and dark regions, whereas Cæcidotea only occasionally reacts at that plane.

III. ILLUMINATION BY DIRECT SUNLIGHT WITH THE RAYS AT RIGHT ANGLES TO THE LONG AXIS OF THE TANK

As in the experiments with vertical illumination, the tank was so arranged in this series as to have a dark and an illuminated region with a sharp plane of separation between the two. This series of experiments was made at a south window in the basement of the Museum. The available space directly within this window was large enough to enable one to make use of the direct sunlight for only a limited period (about two hours daily), but fortunately this period came at about mid-day. Just what the sun's luminosity ordinarily is at mid-day in Cambridge was not determined. Naturally so many factors affecting the luminosity are concerned that any determination or estimate of it at one time would have little value for any other time. To my eye the brightness at the tank under direct mid-day sunlight ordinarily seemed approximately twice as great as that produced by the 6983 C.M. artificial light so often used. In these experiments the inner glass tank filled with water to a depth of 3 cm., was used without the outer tank. Under one half of the tank was placed black cloth of several thicknesses. This extended exactly to the middle of the tank, and was fitted to the end and sides and over the top of that half. To further exclude the light from this part of the tank, a black card-board partition extended down from the edge of the cloth at the median plane. This partition just cleared the surface of the water, so that the only light which entered the dark end came through the water below the partition. Of course the so-called dark end was considerably illuminated owing to the diffusion of light through the water but the plane between the illuminated and dark regions was nevertheless very sharp, so that the contrast between the two was strong. The tank was placed upon a light but rigid box so that it could

be shifted occasionally in order to keep the sun's rays approximately at right angles to the long axis of the tank. This mechanical disturbance, while exceedingly undesirable, was non-directive in its effects. It did, however, tend to increase the activity of the animals and thus indirectly increased the average percentage in the illuminated region, rather than that in the dark region. Furthermore, since the shifting did not occur oftener than every 15 minutes, and since within fifteen minutes after the experiment started practically the maximum response had occurred, the results were already virtually attained before any shifting was necessary.

In these experiments the temperature of the water rose rapidly, but since the water used was 3 cm. in depth and the sun's rays entered it somewhat obliquely, they passed through a thickness of more than 3 cm. of water. Hence it is safe to say that heat was not the effective stimulus in these experiments.

1. Asellus

The following table (XIII) shows the results of one of these experiments, in which 43 Asellus communis were used. After obervations at intervals of sixty seconds for about half an hour, at 11:01 the illuminated end of the tank was quickly darkened and the one previously dark was suddenly illuminated. At 11:30 a return to the initial illumination was made.

This table shows that Asellus very promptly avoids direct sunlight under the conditions of these experiments. The animals in the illuminated region showed great activity. This appeared very promptly with most individuals, nearly all beginning to move within one minute after the sunlight was allowed to reach them. This soon resulted in bringing all the individuals into the dark area. In no case did it require more than about five minutes for nearly all the animals to find their way into the dark region; once there, very few came back at all. In most cases those which did come back remained in the sunlight for only a minute or even less. After each disturbance caused by shifting the tank to compensate for the earth's motion, several usually came out into the illuminated region; but they remained in the light only a

TABLE XIII

ASELLUS COMMUNIS (43 individuals). October 12, 1906

Dark and illuminated regions normal			*Dark and illuminated regions reversed*		
TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION	TIME OF MAKING RECORDS	NUMBER IN DARK REGION	NUMBER IN ILLUMINATED REGION
			11:03	0	43
10:34	26	17	11:04	15	28
10:35	5	38	11:05	25	18
10:36	4	39	11:06	36	7
10:37	2	41	11:07	41	2
10:38	1	42	11:08	41	2
10:39	0	43	11:09	41	2
10:40	0	43	11:10	40	3
10:41	0	43	11:11	42	1
10:43	3	40	11:12	42	1
10:44	1	42	11:13	42	1
10:45	0	43	11:14	43	0
10:46	2	41	11:15	43	0
10:47	0	43	11:16	42	1
10:48	0	43	11:17	40	3
10:49	1	42	11:18	41	2
10:50	3	40	11:19	41	2
10:51	0	43	11:20	43	0
10:52	0	43	11:21	43	0
10:53	0	43	11:22	43	0
10:54	0	43	11:23	43	0
10:55	0	43	11:24	42	1
10:56	0	43	11:25	43	0
10:57	1	42	11:26	43	0
10:58	1	42	11:27	42	1
10:59	1	42	11:28	43	0
11:00	0	43	11:29	43	0
11:01	0	43	11:30	43	0
Averages for whole time	1.9−, or 4.4%	41.1+, or 95.6%	Averages for whole time	39.2−, or 91%	3.8 +, or 9%

TABLE XIII—(*Continued*)

Dark and illuminated regions returned to normal

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION	TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARK REGION
11:33	39	4	11:53	0	43
11:34	10	33	11:54	0	43
11:35	6	37	11:55	0	43
11:36	1	42	11:56	0	43
11:37	1	42	11:57	0	43
11:38	1	42	11:58	0	43
11:39	2	41	11:59	0	43
11:40	2	41	12:00	0	43
11:41	1	42	12:01	0	43
11:42	1	42	12:02	0	43
11:43	2	41	12:03	0	43
11:44	0	43	12:04	0	43
11:45	0	43	12:05	0	43
11:46	1	42	12:06	0	43
11:47	0	43	12:07	0	43
11:48	0	43	12:08	0	43
11:49	0	43			
11:50	0	43	Averages for		
11:51	0	43	whole time.	1.8 +, or 4.3%	41.2−, or 95.7%
11:52	0	43			

very short time. No careful study of the animal's actions at the bounding plane between the illuminated and dark regions was attempted, but numerous instances were noted in which animals started into the illuminated area and at once turned back.

Several other experiments upon Asellus under the influence of direct sunlight were made and the results were fully as striking as in the observations tabulated above. On the average for the whole time of the experiments of this series only 9.6 per cent of the animals were in the illuminated area.

By way of summary it may be said that Asellus was extremely sensitive to direct sunlight, which it avoided if opportunity was afforded, and that immediately upon entering the illuminated area it often turned back.

2. *Cæcidotea*

Under the same conditions of illumination several experiments were made with Cæcidotea. The results of one of these, in which 21 individuals were employed, are shown in detail in Table XIV.

This table shows that under the conditions of illumination described, Cæcidotea, like Asellus, tended to collect in the dark region. The photokinetic effect appeared quite promptly with the animals in the illuminated region, though it was less prompt than with Asellus. However, most of the animals began moving within from three to five minutes, and within eight minutes this activity had led a majority of them into the dark region. After entering the dark region they returned to the illuminated one much more than Asellus did; but even with Cæcidotea the number which kept returning was comparatively small. The number which started into the illuminated region and turned back within a few seconds was such as to suggest that the influence of the change in illumination at the plane of division between the two regions was in many cases immediate. A more careful study of this point seemed desirable, but an unobscured sun at mid-day came so seldom at that time, that the necessary observations were not made.

The other experiments made with Cæcidotea under the same conditions gave results in entire accord with those of the experiment discussed above. The average per cent of Cæcidotea in the illuminated area for the whole time of all the experiments of this series was 22.4.

Cæcidotea, then, was quickly affected in a photokinetic way by direct sunlight, though not so quickly as Asellus. This activity incidentally led the animal into the dark region, from which it very generally did not return. Cæcidotea just entering the illuminated region from the dark one sometimes turned back into the dark region at the plane of division between the two regions.

Comparing Asellus with Cæcidotea when subjected to illumination by direct sunlight with the rays at right angles to the long axis of the tank, Asellus proved decidedly the more responsive. It was affected more quickly by the sunlight, sooner

TABLE XIV

Cæcidotea stygia (21 individuals)
October 3, 1906

TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARKENED REGION	TIME OF MAKING RECORDS	NUMBER IN ILLUMINATED REGION	NUMBER IN DARKENED REGION
10:56	11	10	11:45	1	20
10:57	10	11	11:46	2	19
10:58	7	14	11:47	2	19
10:59	7	14	11:48	1	20
11:00	8	13	11:49	2	19
11:01	5	16	11:50	2	19
11:02	6	15	11:51	2	19
11:03	6	15	11:52	2	19
11:04	4	17	11:53	2	19
11:05	3	18	11:54	1	20
11:06	7	14	11:55	1	20
11:08	10	11	11:56	0	21
11:09	10	11	11:57	1	20
11:10	7	14	11:58	2	19
11:11	7	14	11:59	1	20
11:12	4	17	12:00	1	20
11:13	3	18	12:01	2	19
11:14	6	15	12:02	3	18
11:15	8	13	12:03	4	17
11:16	4	17	12:04	5	16
11:17	5	16	12:05	6	15
11:18	5	16	12:06	3	18
11:19	5	16	12:07	3	18
11:20	2	19	12:08	3	18
11:21	2	19	12:09	3	18
11:23	4	17	12:10	3	18
11:24	5	16	12:13	0	21
11:25	6	15	12:14	0	21
11:26	5	16	12:15	2	19
11:27	3	18	12:16	2	19
11:28	4	17	12:17	2	19
11:29	4	17			
11:30	2	19	Averages for		
11:31	2	19	whole time.	3.8+, or 18.+%	17.2−, or 82.−%
11:32	3	18			

entered the dark region, and less often returned to the illuminated one.

A general discussion of these results is reserved for the second part of this paper.

IV. SUMMARY OF REACTIONS TO LIGHT

1. With horizontal illumination

1 Asellus communis exposed to horizontal illumination is not responsive to intensities of light of 1 C.M. or less.

2 It is very decidedly affected in a photokinetic way by those light intensities to which it responds.

3 It is also affected in a phototactic way by horizontal illumination. Following exposure to such light, it is negative to an intensity of 2.5 C.M. or more. It is neutral to an intensity of 1 C.M. or less. After retention in darkness for a few hours, it is positive to such intensities as call forth any response (2.5 C.M. or more); but to an intensity of 2855 C.M. the positive response is only momentary.

4 Its response appears to be direct, being produced by the effects of unsymmetrical stimulation of the two eyes of the animal.

5 Cæcidotea stygia is not responsive to light intensities below about 80 C.M. It is negative to such intensities as it responds to at all—80 C.M. or more.

6 This response of Cæcidotea is photokinetic in its nature, the random movements causing the animals ultimately to settle in the negative end of the tank, where the intensity of illumination is least; to this intensity the animals soonest become acclimated.

7 After considerable exposure to strong light both Cæcidotea and Asellus become less reactive to it. Conversely, following retention to darkness they are both apparently somewhat more responsive to light.

11. With Vertical Illumination

With one half of the tank illuminated by light of 6983 C.M. intensity falling vertically upon it, and with the other half as

dark as it could be made under the conditions, there being a sharp plane of demarkation between the halves:

1 Asellus is more active in the illuminated region than in the dark one.

2 The photokinetic effect upon Asellus is such that for a time it very generally recoils from the end of the darkened half of the tank and reënters the illuminated region.

3 Asellus shows immediate responsiveness to the sudden change in the intensity of illumination, these responses manifesting themselves in inhibition of the animal's movements. Sometimes it stops on entering the light region—whereupon it occasionally turns back, but more often this happens when it first enters the dark region. Sometimes Asellus shows immediately accelerated movements when it enters the illuminated region, but when this is not the case increased activity usually appears very soon.

4 The animals (Asellus) ultimately collect and remain within the dark region.

5 The reactions of Cæcidotea resemble those of Asellus, but Cæcidotea reacts at the bounding plane much less often and less decidedly than Asellus. Cæcidotea collect within the dark region more slowly than Asellus and do not remain there as exclusively as do the latter animals.

III. With Illumination by Direct Sunlight

To the sun's rays at right angles to the long axis of the tank both species are more responsive than to an illumination by artificial (Nernst lamp) light of 6983 C.M. intensity, although they respond in much the same manner. As under the other methods of illumination, Asellus is decidedly more responsive than Cæcidotea.

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STUDIES IN THE LIFE CYCLE OF HYDATINA SENTA

I. ARTIFICIAL CONTROL OF THE TRANSITION FROM THE PARTHENOGENETIC TO THE SEXUAL METHOD OF REPRODUCTION.

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INTRODUCTION

In the attempt to solve the problem of sex determination, important experimental evidence supposed to bear on the question has been derived from the rotifer Hydatina senta. It seems probable, however, that the phenomena referred to in Hydatina do not bear on sex-determination. It is practically certain that the mother of the males is the sexual female. What at first seemed, therefore, a question of sex-determination relates instead to the transition from the parthenogenetic to the sexual phase of the life cycle, so that one could substitute the term sexual female for the term male-producer in describing the phenomena. The

important problem in Hydatina, therefore, is to determine what conditions, whether external or internal, bring about the change from the parthenogenetic to the sexual mode of reproduction, and so affect the proportion of male-producers. With a view to testing anew the influence of the various agents which have been supposed to alter this proportion, the experiments described in this paper were performed. The results of the experiments do not support the view that any of the proposed agents have a direct influence on the proportion of male-producers; but they do give evidence of another factor, not hitherto suggested, a factor which not only accounts for the results of the present experiments, but affords a simple probable explanation of the results upon which the previous contradictory conclusions were based. The results of the experiments indicate that the presence of certain substances dissolved in the water in which the rotifers are reared may exert a potent influence on the proportion of male-producers; and they make it probable that the agents formerly thought to exert such an influence either have no influence at all, or exert it only indirectly by first affecting the dissolved substances. The previous contradictory conclusions may thus be brought under a common point of view. The above conclusion regarding dissolved substances was reached only after a number of tests had been made of the factors previously believed to influence the proportion of male-producers; in the account given in this paper, the experiments are described approximately in the order in which they were performed.

The life cycle of Hydatina senta is as follows: From the fertilized or resting egg there hatches invariably a female. This female may, under favorable circumstances, produce parthenogenetically 40 to 50 offspring, all of the same sex, which so far as I have observed is always the female. These females may in turn produce 40 to 50 young, all the offspring of one parent being of the same sex; but while some of the females produce females, others may produce males. Thus the females may be spoken of as male-producers or female-producers. The number of male-producers in a family varies within wide limits; it may be zero, or it may be 100 per cent. When males have appeared in a colony, resting

eggs may also appear provided the young females copulate within a few hours after hatching. The winter eggs are recognizable by their large size, and thick shell which is covered with many processes like the nap or pile of a fabric. The parthenogenetic eggs have thin shells; those yielding females are usually larger than those hatching into males, but eggs of intermediate size may be of either sex.

Most of the above facts were reported by Maupas ('90a). In later investigations, the same author (Maupas ('90b) found that when young females were given every chance to copulate, no males were produced and about the same percentage of females produced winter eggs as produced males when copulation was prevented. From this he concluded that the winter or resting eggs are fertilized male eggs, and that young females destined to produce females can not be fertilized. Maupas ('91) also performed experiment from which he concluded that the proportion of male-producers depends on temperature. The offspring of five females kept at 26° to 28° C. included 97 per cent of male-producers, while five sister females at 14° to 15° C. yielded only 5 per cent of male-producers. Five other females which were kept in the cold while they laid the first half of their output of eggs, and at 26° to 28° C. while laying the last half, yielded 24 per cent of male-producers in the former lot, and 81 per cent in the latter. Six other females were alternated between high and low temperature, and the highest percentage of male-producers came from eggs laid at the high temperature.

The possible influence of temperature was afterwards examined by Nussbaum ('97), who got only negative results with temperature differences. Nussbaum's experiments showed, he thought, that starvation increased the proportion of male-producers. He tried to reconcile Maupas's findings with his own as follows: Maupas probably did not isolate the young rotifers as they hatched, so that his aquaria soon came to contain many individuals. At the higher temperature, the animals multiply so much more rapidly and each one eats so much more, that the quantity of food put into the dishes at the outset soon became exhausted. The ensuing starvation, Nussbaum supposed, effected the increase in

the number of male-producers. Temperature, according to his explanation, was only indirectly responsible for the change. He further suggested that Maupas may have determined the sex of the offspring by the size of the egg, which, as Nussbaum himself first pointed out, is not a safe criterion. Nussbaum believed that these two explanations, together with "chance," sufficiently accounted for Maupas's remarkable results.

It will be profitable to state briefly the evidence which led Nussbaum to the above conclusion regarding starvation. His experiments are open to doubt on the ground that they were not usually controlled. Possibly some experiments were controls of others where it is not apparent from the text. Nussbaum rarely mentions controls, hence it is probable that in most cases he did not intentionally institute them. His general method seems to have been to raise the rotifers under such circumstances as he could provide, watch the course of the experiments, and record the changes, such as scarcity of food, gradual change of temperature, etc., that occurred. In certain temperature experiments, however, the controls were definitely maintained.

When an aquarium showed signs of scarcity of food and males afterwards appeared in it, the experiment was taken as evidence of the influence of starvation. Evidence of scarcity of food was of four kinds: (*a*) The presence of many females in the same aquarium, in which case there must have been less food for each one, even if scarcity was not otherwise apparent; (*b*) a low rate of egg production; (*c*) partial emptiness of the gut of the animals; (*d*) direct observation of the food. There were about eighty experiments in all. It is often difficult to decide whether starvation occurred, for there were all degrees from starvation to good feeding. I have tried to examine the published data impartially with the following results.

1. In 13 experiments many females were left in one aquarium (from which it might be supposed that the food supply for each was deficient), and males appeared; and in four experiments where distinctly few females occupy the same aquarium, no males appeared. But in at least one other experiment (54), many females lived together without producing males.

2. In three experiments a rather low rate of egg production (from which partial starvation was inferred) was followed by the appearance of males.

3. In 12 experiments, where abundant food was supplied and the experiment continued for several days, only females were produced.

4. In seven experiments, where hunger was evidenced by the small quantity of free food present or the state of fullness of the gut, males appeared later; but in seven other experiments hunger was not followed by the production of males, and in five others males appear without preceding hunger.

It appears that Nussbaum draws his chief support from the cases of inferred starvation in (1) and (2) above, since those in (3) and (4) are contradictory. As the rate of egg-production varies considerably even with abundance of food, the conclusion that starvation increases the proportion of male-producers rests largely upon the cases where the food of a single aquarium was divided among many individuals.

The conclusions of Maupas and Nussbaum were tested by Punnett ('06) in several experiments carried out with great care. He isolated each young female and followed its history individually, which neither of the preceding investigators seems to have done. He was unable to secure in three generations an increase in the proportion of male-producers by starving the young females for some hours after hatching. Variations of temperature from 8° to 23° C. yielded no results, though the animalswere kept four to eight days near each extreme. Punnett thought he found evidence, however, of strains, each yielding a rather definite proportion of male-producers. He recognized three types of parthenogenetic female; one yielding many male-producers (ca. 40 per cent), one few male producers (ca. 2 per cent), and one no male-producers. His general conclusion was that external conditions had no influence on the sex of the offspring, but that this was determined by an internal factor, the zygotic constitution. The character of the male and female elements uniting in the winter egg would, according to his interpretation, determine the ratio of male-producers in the parthenogenetic generations that fol-

lowed, and that ratio would be fairly constant regardless of external conditions, until the parthenogenetic series was terminated.

Whitney ('07) made more extensive experiments, including several thousand individual records, which sustained Punnett's conclusion that neither temperature nor food influenced the percentage of male-producers. He found no evidence, however, of constant strains, for he was able to derive lines yielding many male-producers from lines yielding few, and *vice versa*. He attempted to explain Maupas's results in two ways. First, he found that at the high temperature used by Maupas (26° to 28°C.), male-producers laid from two to four times as many eggs as did female-producers. Maupas probably assumed that the number of eggs was approximately the same for each, and in this way, Whitney concludes, introduced an error. Second, Whitney found that the male-producers appeared chiefly in the early part of the family, and since the high temperature used by Maupas reduced the size of the families, the proportion of male-producers was accordingly raised. That the first explanation is invalid will be shown later. On the second point, some light is thrown by data given in this paper.

In view of these conflicting results, it seemed highly desirable that the whole question be reëxamined. I undertook the work at the suggestion of Prof. T. H. Morgan, and I am indebted to him for suggestions and encouragement throughout.

PROBLEM AND METHODS

The problem in Hydatina, briefly stated, was to discover the factor or factors, either external or internal, which determine the proportion of male-producers.

In the experiments to be described, each female was isolated in a Syracuse watch-glass, and kept in about 2 cc. of Great Bear Spring water. The food used was chiefly a colorless flagellate, Polytoma uvella. I have had much better success with this than with Euglena or its allies. It was originally secured from a small stream containing kitchen drainage on the Palisades in Grantwood, N. J., in June, 1909, and has been readily propagated from one

culture to the next since that time. Cultures were made by immersing fresh horse-manure, tied up in cheese-cloth, in about three times its volume of water. Manure giving a rich brown solution gave much better results than paler solutions. After the manure had been extracted for one to several days, Polytoma uvella from an older culture was introduced. In from one to five days it was abundant enough to use. The quantity of water in a food culture varied from one pint to several quarts; the smaller cultures were ready to use earlier, and also passed their optimum in shorter time. Cultures were made up fresh at intervals of one to several days; no culture was found to last satisfactorily longer than four or five days, usually less.

Pipettes used in handling the young females were heated in a gas or alcohol flame before using a second time. Pipettes used for food cultures were never used in transferring rotifers; at no time in all my work were any rotifers of this species found in the food cultures. In all experiments performed at Columbia University, the Syracuse watch-glasses were heated in an oven to a temperature of about 200° C. before being used again. In work done at Cold Spring Harbor, L.I., the dishes containing females from which I was breeding had been placed in boiling water for several minutes after previous use. Dishes for individuals to be kept only until the sex of their offspring was determined, and then discarded, were washed carefully and allowed to dry thoroughly, but not heated. In the course of the summer, 300 of these dishes were tested by placing in them water and food, but no rotifers. Not a single rotifer ever appeared in any of these dishes. Furthermore, had there been any adhering rotifers or eggs, these would have appeared later when the records were made. Had not the presence of a foreign rotifer been evident from the nearly equal size of two of them, it must frequently have occurred in a series of families producing many males, that the two rotifers yielded offspring of different sexes. Out of over nine thousand records from unheated dishes, there was not one case of this kind. I conclude, therefore, that no error has been introduced by failure to heat dishes before a second using.

TABLE I

Showing number of male- and female-producers in a series of 81 generations of Hydatina senta bred under the most favorable circumstances attainable. Male-producers are designated ♂♀, female-producers ♀♀.

NO. OF GENERATION	DATE OF FIRST YOUNG	NO. OF ♂♀	NO. OF ♀♀	NO. OF GENERATION	DATE OF FIRST YOUNG	NO. OF ♂♀	NO. OF ♀♀	PER CENT OF ♂♀
1	June 29	19	31	34	Aug. 15	7	45	
2	30	22	25	35	17	3	44	
3	July 2	0	18	36	19	0	41	
4	3	30	13	37	20	0	44	
5	5	26	9	38	22	0	7	
6	6	23	16		22	0	35	
7	8	41	9	39	23	0	25	
8	9	38	16	40	25	6	32	
9	11	31	12	41	26	31	8	
10	12	26	20	42	27	3	28	
11	14	24	14	43	29	20	30	
12	15	5	48	44	30	5	40	
13	16	1	46	45	Sept. 1	29	19	
14	17	4	46	46	3	28	10	
15	18	38	13	47	5	13	18	
16	20	9	39	48	8	0	37	
17	22	41	12	49	10	4	41	
18	23	41	12		10	13	33	
19	25	30	18	50	12	0	44	
20	26	33	17	51	14	9	43	
21	27	23	26	52	16	9*	3*	
22	29	8	28	53	18	0*	1*	
23	30	1	30	54	20	0*	1*	
24	31	10	17	55	22	21	33	
25	Aug. 1	0	8	56	24	6	42	
	1	4	48	57	26	17	14	
26	3	2	19	58	28	28	13	
	3	0	52	59	Oct. 1	25	26	
27	4	0	35	60	3	1	50	
28	6	0	40	61	5	28	15	
29	8	0	42	62	8	37	2	
30	9	2	36	63	10	4	34	
31	10	7	37	64	12	13	32	
32	12	0	39	65	14	2	12	
33	13	0	50	66	15	0	31	

* Remainder of family not recorded.

TABLE I Continued

NO. OF GENERATION	DATE OF FIRST YOUNG	NO. OF ♂♀	NO. OF ♀♀	NO. OF GENERATION	DATE OF FIRST YOUNG	NO. OF ♂♀	NO. OF ♀♀	PER CENT OF ♂♀
67	Oct. 17	0	35	76	Oct. 31	0	14	
68	18	0	48		Nov. 1	8	20	
69	20	10	4	77	2	1	23	
70	21	0	17	78	3	0	43	
71	23	4	27	79	5	4	33	
72	25	9	34	80	8	12	14	
73	27	2	17	81	10	2	5	
74	28	31	13		12	2	9	
75	30	6	32					
Total						992	2262	30.4

EXPERIMENTS

Normal Course of Parthenogenetic Series

Experiment I. A stock of rotifers had been brought into the laboratory about the middle of May and there multiplied rapidly. After being kept in a dish for about four days, the rotifers were nearly all dead, but many winter eggs had been laid. The culture was then placed in a refrigerator at a temperature of 10° to 14° C. until June 26, excepting three days from June 16 to 19. On June 26 it was brought to room temperature, and on June 27 a young female was isolated from it. From this female was bred a series of 81 generations under what seemed to be the best obtainable conditions. The first member of each family became, when possible, the parent of the next generation. Usually only one family was reared in each generation.

The purpose of this experiment was to ascertain the nature of the fluctuations in percentage of male-producers, which might occur without intentional alteration of the conditions by the experimenter. The results are shown in Table I.

The data show that, besides the considerable fluctuation in the percentage of male-producers which appears between one generation and the next, there may also be long-continued periods in which few male-producers appear, followed by equally long periods in which they are abundant. The generations between one winter egg and the next do not behave as a strain having a fairly constant proportion of male-producers.

Variability of the Percentage of Male-producers in Related Strains under Like Conditions

Experiment II. To determine what difference in the percentage of male-producers must be obtained to give positive indications of the influence of a given agent, two series of generations were reared under like conditions. Both series were reared in the same water, at the same temperature, and were fed approximately equal amounts of the same food cultures. The experiment was performed twice, A and B, Table II. In A, the parents of the two series were first cousins once removed; in B, fourth cousins.

The difference in the first experiment is about six per cent, in the second less than 2 per cent. In the former case, where the difference between the two percentages of male-producers is greatest, the ratio of the higher to the lower percentage is about 1.1 to 1.0. In experiments designed to test the influence of a given agent, unless the ratio of the higher to the lower percentage of male-producers is greater than 1.1 to 1.0, it is not safe, therefore, to infer from a single experiment that the agent in question has any influence; and the greater this ratio, the stronger is the evidence of such influence. In case of an agent having but slight effect, this effect should be shown by numerous experiments giving small differences of practically uniform sign.

Influence of Quantity of Food Culture on Percentage of Male-producers.

Experiment III. On July 22 two sister individuals, respectively the first and second of their family, from the 17th generation

of Experiment I., were isolated. One with its progeny was abundantly fed (five to ten drops of the culture) on what was considered the best food. The other with its progeny was fed only so much as was judged would be necessary to maintain life and enable them to produce a moderate family. The shorter families indicate that partial starvation actually occured, but this starvation

TABLE II

Showing the number of male- and female-producers in two series of generations from related parents, both series being reared under like conditions.

Experiment	No. of Generation	Series I				Series II			
		Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
A.....	1	July 2	31	21		July 2	0	18	
	2	3	27	15		3	30	13	
	3	5	23	15		5	26	9	
	4	6	25	26		6	23	16	
	5	8	34	16		8	41	9	
	6	9	36	12		9	38	16	
	7	11	24	22		11	31	12	
	8	13	23	26		12	26	20	
Total.....			223	153	59.3		215	113	65.5
B.....	1	July 22	26	27		July 22	41	12	
	2	24	15	34		23	41	12	
	3	26	28	16		25	30	18	
	4	27	13	31		26	33	17	
	5	28	18	14		27	23	26	
	6	29	10	22		29	8	28	
		31	5	7					
	7	31	25	15		30	1	30	
		31	0	3					
	8	Aug. 1	3	33		31	10	17	
	9	3	7	29		Aug. 1	0	8	
						1	4	48	
	10	4	0	39		3	2	19	
						3	0	52	
	11					4	0	35	
Total.....			...150	270	35.7		193	322	37.4

TABLE III

Showing number of male- and female-producers in a series of 55 generations of Hydatina senta which were well fed, and a series of 54 generations which were starved.

Well-fed					Starved.				
No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
1	July 23	41	12		1	July 23	27	23	
2	25	30	18	63.5	2	25	20	5	60.5
3	26	33	17			25	13	12	
4	27	23	26		3	27	12	7	
5	29	8	28		4	28	20	7	
6	30	1	30		5	29	14	6	53.6
7	31	10	17	14.9	6	31	3	11	
8	Aug. 1	0	8		7	Aug. 1	0	8	
	1	4	48		8	3	0	3	
9	3	2	19			3	20	10	29.8
	3	0	52	1.3	9	4	3	11	
10	4	0	35		10	6	0	30	
11	6	0	40		11	8	15	14	
12	8	0	42		12	9	1	8	26.5
13	9	2	36	7.2	13	11	1	25	
14	10	7	37		14	12	6	25	
15	12	0	39		15	14	10	11	25.0
16	13	0	50	4.9	16	16	0	12	
17	15	7	45		17	18	14	19	
18	17	3	44		18	19	9	26	30.2
19	19	0	41	2.2	19	21	0	8	
20	20	0	44		20	22	0	7	
21	22	0	7		21	24	9	24	37.8
	22	0	35		22	26	16	10	
22	23	0	25	25.6	23	27	15	12	
23	25	6	32		24	29	5	23	37.6
24	26	31	8		25	30	9	13	
25	27	3	28		26	Sept. 2	6	16	
26	29	20	30	22.2	27	4	4	6	36.8
27	30	5	40		28	6	11	14	
28	Sept. 1	29	19		29	8	2	14	
29	3	28	10	59.8	30	10	10	23	16.0
30	5	13	18		31	12	0	26	
31	8	0	37		32	13	1	15	.
32	10	4	41		33	15	10	6	33.3
	10	13	33	9.8	34	17	0*	1*	
33	12	0	44						

*Remainder of family not recorded.

TABLE III—continued

Well-fed					Starved				
No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
34	Sept. 14	9	43		35	19	o*	1*	
35	16	9*	3*	27.6	36	Sept. 21	o*	1*	
36	18	o*	1*		37	23	o	6	41.1
37	20	o*	1*		38	24	14	12	
38	22	21	33	26.2	39	26	12	26	
39	24	6	42		40	28	9	17	32.8
40	26	17	14		41	Oct. 1	2	6	
41	28	28	13	62.5	42	3	1	27	35.3
42	Oct. 1	25	26		43	5	20	9	
43	3	1	50	37.2	44	8	13	20	
44	5	28	15		45	10	7	18	39.7
45	8	37	2		46	11	15	15	
46	10	11	34	47.2	47	13	12	12	
47	12	13	32		48	16	o	18	
48	14	2	12		49	17	2	38	28.5
49	15	o	31		50	18	19	13	
50	17	o	35	1.5	51	20	5	24	
51	18	o	48		52	22	17	25	
52	20	10	4		53	23	16	19	40.2
53	21	o	17		54	25	16	12	
54	23	4	27	21.9					
55	25	9	34						
Total		553	1652	25.0			466	810	36.5

*Remainder of family not recorded.

continued throughout life, and was not concentrated in the first few hours after hatching. The results are shown in Table III.

There is a decidedly greater proportion of male-producers among the starved generations than among the well-fed. Since there is considerable fluctuation in the well-fed line, covering long periods, it is instructive to divide the experiment into parts, as shown in Table IV.

Had the experiment ended with the family started on July 27, or had it included only the period from August 26 to September 5, or from September 21 to October 12, there would have been

no very clear evidence of any influence of food on the proportion of male-producers; what evidence there is in these parts of the experiment would have gone to show that starvation reduced the percentage of male-producers. But in those parts of the experiment from July 28 to August 25, and from October 13 to October 25 the evidence is very decisive on the other side; and the period from September 6 to September 20, while not so marked, also points strongly to the conclusion that starvation is accompanied by an increase in the proportion of male-producers.

TABLE IV

A summary of the data given in Table III, dividing it into periods according to the percentage of male-producers in the well-fed series.

Limiting Dates	Well-fed			Starved		
	no. of ♂♀	no. of ♀♀	per cent of ♂♀	no. of ♂♀	no. of ♀♀	per cent of ♂♀
July 23 to July 27	127	73	63.5	72	47	60.5
July 28 to Aug. 25	50	754	0.6	125	265	32.0
Aug. 26 to Sept. 5	129	153	45.7	55	80	40.7
Sept. 6 to Sept. 20	35	203	14.7	34	100	25.3
Sept. 21 to Oct. 12	187	261	41.7	93	157	37.2
Oct. 13 to Oct. 25	25	208	10.7	87	161	35.0

These apparently conflicting results do not show that the food culture nas no influence on the proportion of male-producers. It is to be noted that the proportion of male-producers in the starved line is much more constant than in the well-fed line. The change in the proportion of male-producers from one group to the next is always of the same sign in the starved series as in the well-fed, but is less in amount. This greater constancy of the starved families is well shown in the figure (see opposite page), in which the percentage of male-producers in each series is represented by a curve. To eliminate the minor fluctuations from one generation to the next, the experiment has been blocked off in five-day periods in July and August, and six-day periods after September 1. The last period includes seven days. The aim was to include

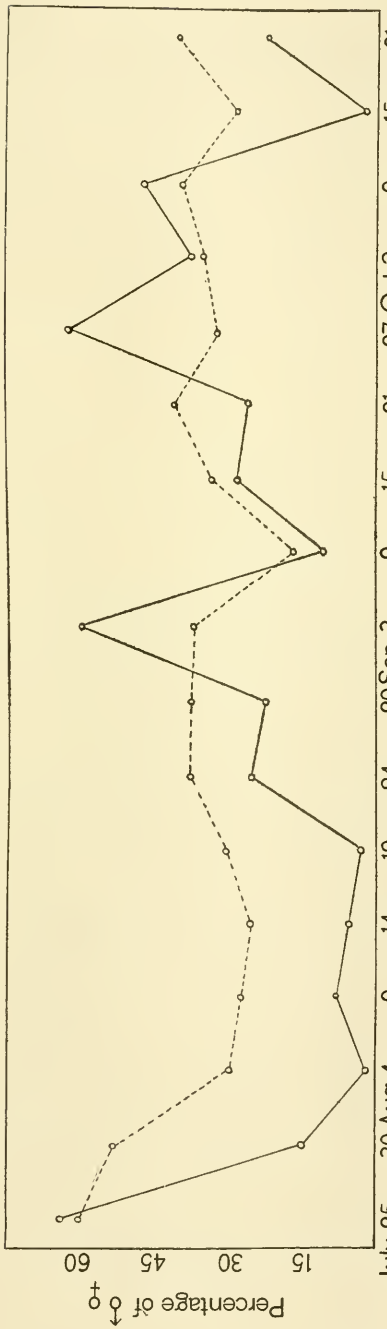

FIG. 1. Curve of the proportion of male-producers in a series of well-fed families, and in a series of starved families. The dotted curve is that of the starved line.

TABLE V.

Showing number of male- and female-producers in three series of generations of Hydatina senta which were abundantly fed; and three control series, derived from sisters, that were starved.

Experiment	No. of Generation	Well-fed: Date of first young	Well-fed: No. of ♂♀	Well-fed: No. of ♀♀	Well-fed: Per cent of ♂♀	Starved: Date of first young	Starved: No. of ♂♀	Starved: No. of ♀♀	Starved: Per cent of ♂♀
A	1	Aug. 18	2	47		Aug. 18	14	19	
	2	20	3	46		19	9	26	
	3	21	0	49		21	0	8	
	4	23	0	30		22	0	7	
	5	25	2	28		24	9	24	
	6	26	34	11		26	16	10	
	7	27	1	41		27	15	12	
	8	29	8	40		29	5	23	
	9	30	7	26		30	9	13	
	10	Sept. 1	9	29		Sept. 2	6	16	
	11	3	11	20		4	4	6	
	12	5	17	27		6	11	14	
	13	7	14	28		8	2	14	
	14	9	12	24		10	10	23	
	15	10	4	34		12	0	26	
	16	12	1	39					
Total			125	519	19.4		110	241	31.3
B	1	Aug. 25	2	28		Aug. 25	0	21	
	2	26	34	11		26	15	12	
	3	27	1	41		27	8	20	
	4	29	8	40		28	12	16	
	5	30	7	26		30	12	11	
	6	Sept. 1	9	29		Sept. 1	14	17	
	7	3	11	20		3	8	10	
	8	5	17	27		5	8	3	
	9	7	14	28		7	13	17	
	10	9	12	24		9	19	10	
	11	10	4	34		11	3	26	
	12	12	1	39		13	17	17	
Total			120	347	25.6		129	180	41.7

TABLE V—continued

Experiment	No. of Generation	Well-fed				Starved			
		Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
C.....	1	Aug. 24	3	35		Aug. 24	0	42	
	2	25	0	40		26	8	3	
	3	28	0	44		28	0	2	
	4	29	16	31		29	7	12	
	5	31	8	42		31	9	16	
	6	Sept. 1	10	40		Sept. 2	10	18	
	7	3	17	20		4	23	13	
	8	5	22	4		5	1	7	
Total.....			76	256	22.8		58	113	33.9
Grand total.....			321	1122	22.2		297	534	35.7

about three generations in each period. The two series cannot be compared generation for generation, for the thirtieth generation of the well-fed line occurs five days earlier than the thirtieth generation of the starved line.

All the major crests and depressions of the curve of well-fed families correspond to similar crests and depressions of the curve of starved families; but the curve of the starved line is more nearly uniform, its highest and lowest points are well within the extremes of the curve of well-fed families. This indicates that the same agent is producing the major fluctuations in both lines, but that, that agent operates to a greater degree upon the well-fed families than upon those that were starved. I can find only one factor that meets these requirements, namely, the quantity of the food-culture employed.

Experiment IV. The results of the preceding experiment were controlled by three repetitions of it. In each pair of controls the two lines were derived from sister individuals, one closely following the other in the family.

In A, the parents were derived from the sixteenth generation of the starved line in Experiment III. The best food was used in each.

In B, the parents came from the fourth generation of the well-fed series in A above. In the preceding four generations five male-producers had appeared among 172 female-producers. The best food was used in each.

In C, the parents were derived from a line that had been, for four generations immediately preceding, fed from food cultures that were past their optimum (see Experiment V); during this time one male-producer and 185 female-producers had appeared. The 16 next preceding generations were the first part of the starved line in Experiment III. Food cultures that were past their optimum were used in each line.

The results of these three experiments are recorded in Table V.

The three experiments point to the same conclusion as do those parts of Experiment III which were performed at the same time, namely, that feeding the rotifers a smaller quantity of food increases the proportion of male-producers. This is the case no matter whether the ancestors of the individuals experimented upon had been starved (A), well-fed on fresh food (B), or abundantly fed on old food (C), nor whether the preceding generations included few or many male-producers. It may be noted also that the change in the well fed series in A, from a low to a high percentage of male-producers, occurs almost simultaneously with a similar change in Experiment III. The two lines were only distantly related, but were fed on the same food.

Influence of Age of Food Culture on Percentage of Male-producers

Experiment V. On August 16, two sister individuals from the sixteenth generation of the starved line in Experiment III were isolated and became the parents of two series of generations. Both of these were fed abundantly, one from fresh food cultures, the other from cultures that were deemed to be past their optimum. The old food cultures, were on the average about ten days older than the new cultures. The cultures in use at this time were made up of about three liters of water, and required three to five days after inoculation to reach their optimum. All that can be said with certainty regarding the cultures used in the two lots is that one

TABLE VI

Showing the number of male- and female-producers in the progeny of two sister individuals of Hydatina senta, one line being fed from fresh food cultures, the other from cultures averaging ten days older.

No. of Generation	Fresh Food				Old Food			
	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
1.....	Aug. 18	2	47		Aug. 18	0	50	
2.....	20	3	46		20	0	52	
3.....	21	0	49		21	1	49	
4.....	23	0	30		23	0	34	
5.....	25	2	28		24	3	35	
6.....	26	34	11		25	0	40	
7.....	27	1	41		28	0	44	
8.....	29	8	40		29	16	31	
9.....	30	7	26		31	8	42	
10.....	Sept. 1	9	29		Sept. 1	10	40	
11.....	3	11	20		3	17	20	
12.....	5	17	27		5	22	4	
Total.....		94	394	19.2		77	441	14.8

was invariably considerably older than the other used at the same time. Everyday the various cultures were tested, by growing young females in them, but it was impossible to determine accurately in a short time which culture was the best. The results are given in Table VI.

It appears from the data that the line fed from the old cultures yielded a lower percentage of male producers.

Experiment VI. The preceding experiment was repeated with one modification, beginning August 23 with two females that were related to each other as fourth cousins. These were derived from the line fed on old food in Experiment V, but in this experiment were partially starved, as described in Experiment III, on new and old food cultures respectively. The data are given in Table VII.

The result, as in the preceding experiment, is a smaller proportion of male-producers in the line fed on old food, though the relative difference is smaller.

TABLE VII

Showing the number of male- and female-producers in the progeny of two individuals of Hydatina senta related to each other as fourth cousins, both lines being partially starved, one on new food, the other on old food cultures.

No. of Generation	Fresh Food				Old Food			
	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
1.....	Aug. 24	9	24		Aug. 24	0	42	
2.....	26	16	10		26	8	3	
3.....	27	15	12		28	0	2	
4.....	29	5	23		29	7	12	
5.....	30	9	13		31	9	16	
6.....	Sept. 2	6	16		Sept. 2	10	18	
7.....	4	4	6		4	23	13	
8.....	6	11	14		5	1	7	
Total.....		75	118	38.8		58	113	33.9

Influence of Substances in Water on Percentage of Male-producers

Experiment VII. On June 29 samples of water were taken from the drainage ditch in Grantwood, N. J., where two weeks earlier rotifers and an abundance of green flagellates had been found. At this date, however, no rotifers nor flagellates could be discovered; almost all life, except mosquito larvae, was wanting. The water was somewhat cloudy, as if with soap solution. This water may or may not have contained approximately the same substances as two weeks before. Two parallel lines of rotifers, derived from sister females, were fed on the same food and other conditions were kept the same, except that about eight drops of this drainage was added to each dish in one series, an equal amount of spring water to the other. After nine generations, the conditions were reversed; the line previously reared in dilute drainage was kept in pure spring water, and that previously raised in pure water was then given the usual amount of drainage. Table VIII shows the details of the experiment.

In the first part of the experiment there is a markedly lower percentage of male-producers among those reared in the drainage water. In the second part, the difference is in the same direction but is slight. It should be noted that a similar line in Experiment I, derived from a sister to the parents of the two lines in this experiment, and fed on the same food without drainage water, yielded 60.9 per cent of male-producers from June 30 to July 13, and 30.1 per cent from July 13 to July 19. That decrease in the proportion of male-producers finds a parallel in the left side of Table VIII, but not in the right side.

TABLE VIII

Showing the number of male- and female-producers in the progeny of two sister individuals of Hydatina senta one of which was reared in dilute drainage, the other in pure spring water. The left side of the table is the record of one continuous line, even after the conditions are reversed.

No. of Generation	Pure Water				Dilute Drainage			
	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
1	July 1	7	40		July 1	1	45	
2	2	31	21		2	25	17	
3	3	27	15		3	7	39	
4	5	23	15		5	11	38	
5	6	25	26		6	5	6	
6	8	34	16		8	45	9	
7	9	36	12		9	12	31	
8	11	24	22		11	23	31	
9	13	23	26		12	18	32	
Total		230	193	54.3		147	248	37.2
	Dilute Drainage				Pure Water			
10	July 14	34	14		July 14	16	23	
11	15	11	33		15	2	42	
12	16	7	39		16	30	20	
13	18	11	15		17	21	29	
14	19	8	40		19	15	37	
Total		71	141	33.4		84	151	35.7

TABLE IX

Showing number of male- and female-producers in the progeny of four individuals of Hydatina senta, one line of which was well-fed in dilute drainage, one well-fed in pure water, one partially starved in dilute drainage, the fourth partially starved in pure water.

Experiment	No. of Generation	Well Fed, Pure Water				Well Fed, Dilute Drainage			
		Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
A.....	1	Sept. 5	17	27		Sept. 6	0	42	
	2	7	14	28		8	1	31	
	3	9	12	24		10	20	25	
	4	10	4	34		12	19	27	
	5	12	1	39					
Total			48	152	24.0		40	125	24.2
		Starved, Pure Water				Starved, Dilute Drainage			
B.....	1	Sept. 6	11	14		Sept. 7	0	20	
	2	8	2	14		9	0	33	
	3	10	10	23		10	1	34	
	4	12	0	26		12	7	17	
Total			23	77	23.0		8	104	7.1

Experiment VIII. The preceding experiment was repeated twice, with modifications as follows: Two lines were well-fed, one in dilute drainage, the other in spring water (A, Table IX); two other lines were partially starved, as described in Experiment III, the one in dilute drainage, the other in spring water (B). In each case four generations were reared under the conditions described without recording the sex of the offspring. The subsequent four or five generations are the ones here recorded, so that any effect that is noticeable may be the cumulative effect of eight or nine generations of treatment, instead of the four or five for which the data are given. The parents in A, at the time the drainage water was applied, were sixth cousins, once removed. The parents of the starved lines (B) were sisters.

The starved lines show a decidedly lower percentage of male-producers in the drainage water, but in the well-fed lines there is practically no difference. It is not clear whether this disagreement is due to chance, and indicates that the drainage water has no effect; or whether the more distant relationship of the parents of the well fed lines is responsible for the failure to show different percentages in these lines.

Experiment IX. The influence of substances found in the food cultures, as distinguished from the flagellate used as food, was tested as follows: An old culture, which had been made up with spring water, and which had been rejected about ten days before, was filtered through a Berkefeld filter. The filtrate was

TABLE X

Showing the number of male- and female-producers in the progeny of five sister individuals of Hydatina senta, one line being reared in spring water, the others, in various concentrations of the filtrate from old food cultures.

Spring Water		Old Culture Filtrate							
		one-fourth		one-half		three-fourths		undiluted	
♂♀	♀♀	♂♀	♀♀	♂♀	♀♀	♂♀	♀♀	♂♀	♀♀
12	14	5	37	6	39	4	29	0	46
2	9	3	34	0	22	1	16	0	24
2	11	0	38	0	44	0	29	0	19
0	19	6	34	0	31	0	41	0	20
0	32	1	17	9	31	1	41	0	15
1	31	0	47	0	5	0	36	0	7
2	0	4	40	0	32	0	35	0	30
5	13	1	27	0	42	0	18	0	28
1	20	5	24	0	31	0	16	0	35
1	28	0	42	0	18	2	36	0	19
		0	44	0	34	0	4	0	31
		0	23	0	21	0	27	0	38
						0	34	0	25
Total 26	177	25	407	15	350	8	362	0	337
% of ♂♀	12.8	5.7		4.1		2.1		0.0	

examined with a microscope and found to be free from protozoa. Rotifers were reared in various concentrations of this filtrate, one-fourth, one-half, three-fourths, and undiluted, as well as in pure spring water. The five lines were derived from sisters, and were fed equal quantities of food from the same fresh cultures. The flagellate food lived readily in the filtrate, of all concentrations, and when the records were made two days later it was always abundant. Starvation, therefore, could play no rôle in the results. Table X shows the results.

A comparison of the totals shows that there was a gradual decrease, not only in the percentage of male-producers, but in their absolute number, from the line bred in pure spring water to that bred in the concentrated filtrate.

The three series in dilute filtrate were discontinued at the end of the twelve generations shown in Table X. The line in spring water and that in the undiluted filtrate were bred further, the additional generations in each line being shown in Table Xa.

If the series of generations in the latter table be combined with the corresponding series in Table X, of which they are continuations, it is found that there were nineteen successive generations in the filtrate without a single male-producer. In none of the

TABLE Xa

Continuation of the series of generations bred in spring water and in undiluted filtrate shown in Table X.

No. of Generation	Spring Water		Undiluted Filtrate	
	No. of ♂♀	No. of ♀♀	No. of ♂♀	No. of ♀♀
1	2	23	0	26
2	0	37	0	42
3	0	30	0	35
4	1	20	0	22
5	1	21	0	14
6	0	3*	0	28
7	..	..	0	39*
Total	4	134	0	206
Per cent ♂♀	2.9		0.0	

*Remainder of family not recorded.

previous experiments had I secured more than four or five successive generations of all female-producers.

Experiment X. The preceding experiment was repeated a number of times. In each case, the two control lines were bred from sisters. The old culture filtrate was not diluted in any of these experiments.

In A, Table XI, the sisters were derived from the seventh generation of the line bred in undiluted filtrate in Experiment IX; in B, from the third generation of A above; in C, from a line bred for four generations preceding at room temperature, and for ten generations previous to that at a temperature of 7° to 14° C.; in D, from a line which had been reared for eleven generations at a temperature of 7° to 14° C., and had produced about 38 per cent of male-producers; in E, from the fifteenth generation of the line bred in the undiluted filtrate in Experiment IX; in F, from the second generation of the line in spring water in E above.

Here again the evidence all points to the conclusion that substances found in old food cultures tend to reduce the proportion of male-producers. When the number of male-producers is small, as in some of these experiments, it is necessary to take account of death losses. In Experiment IX, in the line bred in spring water 24 females died without reproducing; in the line bred in concentrated filtrate, 18 were lost in like manner. Had all the lost females in the concentrated filtrate been male-producers, and all those in spring water female-producers, the difference in the proportion of male-producers between the two lines would not even then be entirely obliterated. That such selective deaths should occur is not likely, for in starting a series of generations in the old culture filtrate, many young females were put into the concentrated filtrate within one or two hours after hatching, and some of these produced males. They and their male offspring seemed perfectly healthy. I conclude therefore, that the losses by death are as likely to be from among the female-producers as among the male-producers. Moreover, in Experiment X, A, there were only two losses in the filtrate, as compared with 33 male-producers in spring water; and in D, only 4 females were lost in the filtrate, as against 49 male-producers in spring water. In these two experiments (X, A and D), the death losses are entirely insignificant.

TABLE XI

Showing the number of male- and female-producers in the progeny of sister individuals of Hydatina senta' one line being bred in spring water, the other in undiluted filtrate of old food cultures.

	Spring Water					Old Culture Filtrate				
Experiment	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
A.....	1	Nov. 20	16	27		1	Nov. 20	0	35	
	2	21	6	41		2	21	0	19	
	3	23	4	38		3	23	0	31	
	4	25	5	36		4	25	0	38	
	5	27	1	31		5	27	0	25	
	6	29	1	43		6	28	0	26	
Total.....			33	216	13.2			0	174	0.0
B.....	1	Nov. 25	5	36		1	Nov. 25	0	41	
	2	27	1	31		2	27	0	31	
	3	29	1	43		3	28	0	35	
Total.....			7	110	5.9			0	107	0.0
C.....	1	Dec. 1	2	44		1	Dec. 1	0	25	
	2	3	1	28		2	3	0	12	
	3	5	9	26		3	5	0	17	
	4	7	5	17			5	0	2	
	5	9	6	6		4	7	0	15	
		9	5	28*		5	9	0	28	
	6	11	16	14*		6	11	0	22	
							11	0	20*	
Total.....			44	163	21.2			0	141	0.0
D.....	1	Dec. 7	14	25		1	Dec. 6	0	13	
	2	9	14	11		2	8	0	12	
	3	11	17	6*			8	0	12	
		12	2	18*			9	0	23	
		12	2	4		3	10	0	34*	
							10	0	6	
						4	12	0	13*	
							12	0	15*	
Total.....			49	63	43.7			0	128	0.0

TABLE XI—continued

Showing the number of male- and female-producers in the progeny of sister individuals of Hydatinasenta, one line being bred in spring water, the other in undiluted filtrate of old food cultures.

Experiment	Spring Water					Old Culture Filtrate				
	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	No. of generation	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
E.....	1	Dec. 4	2	30		1	Dec. 4	0	22	
	2	7	0	15		2	7	0	14	
	3	8	0	14		3	9	0	28	
	4	9	4	25*		4	11	0	39*	
	5	12	1	22*						
Total.....			7	106	6.1			0	103	0.0
F.....	1	Dec. 6	0	15		1	Dec. 6	0	10	
	2	8	0	14			6	0	16	
	3	9	4	25*		2	8	0	22	
	4	12	1	22*		3	10	0	25	
						4	12	0	9*	
							12	0	3	
Total.....			5	76	6.1			0	85	0.0

*Remainder of family not recorded.

Influence of Breeding From Different Parts of the Family on the Percentage of Male-producers

Experiment XI. Starting June 27 with the individual which became the parent of the series of generations in Experiment I a series of families was bred from the first-born (whenever possible) of each successive generation, and another series from the last-born of each generation. The results are given in Table XII.

A very much greater proportion of male-producers appears among the first-born. To determine whether rearing from the last-born for four generations has any permanent effect in reducing the percentage of male-producers, the offspring of the first member of the last family of last-borns were isolated. Of the family of 48, there were 37 male-producers, or over 77 per cent.

TABLE XII

Showing the number of male- and female-producers in a series of families of Hydatina senta bred from the first member, and another series from the last member of successive generations, all being the progeny of a single individual.

No. of Generation	First-born				Last-born			
	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
1.....	June 30	22	25		July 3	5	20	
2.....	July 2	0	18		8	6	44	
3.....	3	30	13		11	13	41	
4.....	5	26	9		15	2	46	
5.....	6	33	16					
6.....	8	41	9					
7.....	9	38	16					
8.....	11	31	12					
9.....	12	26	20					
10.....	14	24	14					
11.....	15	5	48					
Total.....		276	200	57.9		26	151	14.6

Experiment XII. The preceding experiment was repeated four times. In only a few cases did the first member of a family die without laying eggs, or produce males, and so make it necessary to derive the "first-born" from a later member. Table XIII gives the results in condensed form.

Although in one case the difference in the proportion of male-producers between the first-born and last-born is practically zero, and in two other cases less marked than in the preceding experiment, in no case was there a higher percentage among the last-born. The difference is especially marked where the first-born are yielding many male-producers.

Experiment XIII. The first and fifteenth daughters of one of the females of Experiment I became the parents of two series of generations; one of these was bred successively from the first-born, the other from the fifteenth member, with cer-

TABLE XIII

Showing the number of male- and female-producers in a series of families of Hydatina senta bred from the first-born (whenever possible), and another series from the last-born, of each successive generation.

Date of Beginning Experiment	First-born				Last-born			
	No. of generations	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	No. of generations	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
July 24	8	104	199	34.3	3	10	127	7.2
Oct. 8	9	73	215	25.3	3	26	77	25.2
Oct. 9	9	109	185	37.0	4	22	86	20.3
Oct. 20	5	25	109	18.6	2	6	59	9.2
Total		311	708	30.5		64	349	15.4

TABLE XIV

Showing number of male- and female-producers in a series of families of Hydatina senta bred from the first-born, and another series from the fifteenth-born of each successive generation, all being the progeny of a single individual. A and B are separate experiments.

Experiment	No. of generation	First-born				Fifteenth-born			
		Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀	Date of first young	No. of ♂♀	No. of ♀♀	Per cent of ♂♀
A	1	July 20	9	31		July 21	29	18	
	2	22	41	12		23	8	38	
	3	23	41	12		25	4	45	
	4	25	30	18		27	0	36	
	5	26	33	17		29	3	40	
	6	27	23	26		31	15	9	
	7	29	8	28					
	8	30	1	30					
	9	31	10	17					
Total			196	191	50.6		59	186	24.0
B	1	Aug. 1	0	8		Aug. 2	5	44	
		1	4	48					
	2	2	2	19		5	0	51	
		2	0	52					
	3	4	0	35		7	0	35	
	4	6	0	40		9	0	35	
	5	8	0	42					
	6	9	2	36					
Total			8	280	2.7		5	165	2.9

tain necessary exceptions. The experiment was performed twice, A and B, Table XIV. In A, it was necessary to use one eighth member, and in B one twelfth and one eighteenth member instead of a fifteenth. In B, one second-born was used instead of a first-born.

From these two experiments it appears that breeding from the later parts of the family results in fewer male-producers unless the percentage of male-producers yielded by the first-born is very low.

Influence of Size of Family on Percentage of Male-producers

Whitney ('07, p. 13) endeavored to explain Maupas's high percentage of male-producers in his temperature experiments as due in part to the shortening of the families. He plotted the position of the male-producers in 23 families, and found the great majority of them to appear in the first two-thirds of the family. If the conditions of the experiment curtailed the family by simply omitting the last third of each one, which consisted almost wholly of female-producers, the proportion of male-producers would be greatly increased.

As Whitney's conclusion was based on a small number of families, I have collected data from 349 families, varying in size from 11 to 56, comprising about 12000 individuals, bred during the summer and early autumn of 1909. Table XV groups this data according to the size of the family.

An examination of any one of these groups of families plainly shows that there is no accumulation of male-producers near either end of the family. The small numbers in the last four places in each group are in part due to the fact that most of the families did not reach the maximum length of their respective groups.

It is conceivable that the difference in the proportion of male-producers caused by starvation is referable to the shortened families. In general, partial starvation was found to increase the proportion of male-producers. It might be supposed that the number and position of the male-producers in the family was predetermined; if the middle third of the family were destined to be

largely male-producers, the mother would be able notwithstanding the small quantity of food to prolong her family to include all the male-producers. But if the middle third of the family were destined to be chiefly female-producers, then partial starvation would prevent this middle third from being produced at all. If this be the true explanation of the higher percentage of male-producers in the starved families, then those families which reached more than the average length should show an accumulation of male-producers in their latter portions. There were 76 starved families in my experiments, including 1876 members, or an average of 24.6 per family. Of the 41 families that contained more than

TABLE XV

Showing the number of male-producers occupying the various places in their respective families, compiled from 340 families.

SIZE OF FAMILY	NO. OF FAMILIES	NUMBER OF MALE-PRODUCERS OCCUPYING EACH PLACE IN FAMILY																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
51–56.....	32	3	4	6	12	10	13	11	14	10	12	8	8	8	7	9	7	7	9	10	12	12	12	10	13	10	11	13	13
46–50.....	61	6	9	12	13	18	21	18	15	15	15	12	17	16	20	17	21	23	25	22	22	16	22	21	22	20	25	25	26
41–45.....	36	3	2	6	8	4	8	8	8	11	6	7	8	6	9	9	10	11	9	11	12	13	14	14	14	14	13	15	12
36–40.....	35	2	4	7	4	6	5	6	6	8	8	4	4	6	8	11	9	11	14	11	13	9	13	9	8	9	9	10	13
31–35.....	46	3	7	8	8	9	13	12	15	14	12	16	13	16	17	17	19	15	21	20	20	17	18	14	17	10	14	13	9
26–30.....	45	7	8	7	9	10	14	10	13	14	18	16	14	15	17	21	22	19	20	18	18	17	16	16	17	11	7	8	4
21–25.....	31	5	7	9	6	10	12	12	7	11	10	13	12	12	14	10	11	12	11	6	8	6	3	2	2	0			
16–20.....	34	4	5	9	8	8	6	7	8	7	10	2	8	8	10	10	7	4	4	1	0								
11–15.....	29	4	6	5	7	10	8	7	7	6	9	5	1	2	3	2													

SIZE OF FAMILY	NO. OF FAMILIES	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
51–56.....	32	13	13	13	16	15	12	15	14	16	14	15	11	14	12	14	8	8	11	9	8	9	7	7	5	4	3	2	1
46–50.....	61	26	30	28	25	22	24	24	19	21	23	20	20	20	22	15	16	14	11	9	8	4	2						
41–45.....	36	12	15	12	11	14	11	12	14	13	10	11	11	8	11	6	5	1											
36–40.....	35	12	12	10	12	10	8	5	5	5	3	1	0																
31–35.....	46	7	10	5	3	4	3	1																					
26–30.....	45	3	1																										
21–25.....	31																												
16–20.....	34																												
11–15.....	29																												

25 members, 34 lay between 26 and 35 inclusive. The position of the male-producers in these is given in Table XVI.

It is evident, I think, that there is no accumulation of male-producers at either end of these families, and hence that the shortening of the families by starvation is not responsible for the increased percentage of male-producers in partially starved families.

TABLE XVI

Showing number of male-producers occupying the various positions in their respective families, compiled from 34 partially starved families of more than average length.

Size of Family	No. of Families	Position in Family — Number of male-producers occupying each place in family																																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
31–35	13	2	3	4	4	3	4	3	5	6	7	9	8	9	10	10	11	9	10	9	9	8	8	8	6	4	5	4	4	3	0	1	0	1	0	0
26–30	21	4	5	6	6	5	8	7	7	10	12	9	10	9	9	11	12	11	11	11	9	10	9	7	8	6	3	3	2	1	0					

Identity of Sexual Eggs and Male Eggs

Maupas ('90b) inferred from certain numerical relations that the resting eggs of Hydatina senta are fertilized male eggs, and that upon females which are destined to produce females impregnation has no effect. Subsequent investigations have only tended to make the identity of male eggs and sexual eggs more probable, but direct observations to establish the point have been wanting.

Previous workers have all found that each female laid only one kind of egg; (1) parthenogenetic male eggs, or (2) parthenogenetic female eggs, or (3) fertilized resting eggs. If the resting eggs are fertilized male eggs, it would seem possible, by limiting the number of spermatozoa that enter a female during copulation, to secure from her some resting eggs and some male eggs. Since one female can rarely lay over 16 or 17 resting eggs, and usually lays only 10 or 12, even under favorable circumstances, the number of spermatozoa must be less than ten to afford any considerable probability of getting both kinds of eggs from the same female; or at least less than ten must be successful in entering eggs.

Experiment XIV. Over a hundred fertilization experiments were made to test this point. Young females within the first six hours after hatching were placed in several drops of water with a few vigorous males about a day old. They were watched until copulation occurred, then separated by taking them up in a pipette and squirting them vigorously out again. The time of copulation varied from 2 to 25 seconds. But from every one of these females that did not produce females parthenogenetically, I obtained only resting eggs or only male eggs. Some of the fertilized females laid small eggs toward the last of their output, often not any larger than the majority of male eggs; but these eggs had the thick shell and characteristic markings of winter eggs, and they did not hatch even when kept for days, so they were classed as resting eggs.

Later, a number of fertilized females were reared for cytological study. The method of securing them was as follows: A female was isolated and allowed to produce at least one daughter to show that she was a female-producer. There was then placed in the dish with her a male-producer that had been producing young for some hours, together with the 12 to 15 males which had already hatched from her eggs. As the young females hatched in this dish, they nearly all copulated, and were isolated at intervals as as in the other experiments. It thus happened that the male-producer was always about a day older than the female-producer in the same dish, so that the males were old, or sometimes all dead, when the last females of the family hatched. These late females were sometimes not fertilized, and produced males.

On September 9, 1909, a young female, the last member of a family of 46, hatched under the conditions described above, and was isolated. On September 10 she had laid two large eggs, of the shape of resting eggs; but though their shells were thicker than those of parthenogenetic eggs, they were considerably thinner than those of most resting eggs. Without examining them carefully with a microscope, to determine the markings of the shell, I set the dish aside to see whether the eggs would hatch in a few hours, as they probably would if they were parthenogenetic eggs.

By September 11, the same female had laid several small eggs, also with comparatively thin shells. On September 12 there were 23 small eggs in the dish; no more were laid. On September 13, a number of males were swimming in the dish, and some of the small eggs were then only empty shells. Fifteen males in all appeared in the dish, the last two on September 16; the remaining eight small eggs did not hatch.

On September 21 a young female was found in the dish, and one of the large egg shells was broken and empty. The other large egg had not hatched Dec. 17, when it was discarded.

Thus, winter eggs and male eggs were secured from the same parent. Since so far as known all the parthenogenetic eggs of one individual are of the same sex, there seems to be little room to doubt that winter eggs are male eggs that have been fertilized. The bearing of this is pointed out elsewhere.

SUMMARY OF RESULTS

The proportion of male-producers in Hydatina senta may be reduced, even to zero, by rearing the rotifers in the water of old food-cultures, from which the protozoa have been removed. This effect is due to substances dissolved in the water. If, instead of being reared in the water of old food cultures, the rotifers are bred in spring water but fed from old cultures and not fresh ones the proportion of male-producers may be likewise reduced, but in less degree.

Starvation may be accompanied by a higher proportion of male-producers; but this is probably due to the reduced amount of dissolved substances incidentally introduced with the food.

No evidence of so-called "sex-strains" has been found; more or less constant differences attributed to "strains" may have been due to the use of food cultures containing different quantities of dissolved stuffs.

Families bred from the last daughter of a family include on the average fewer male-producers than families bred from the first daughter. This may be due to the accumulation of substances in the water in which the parent was reared.

Male-producers are not more abundant at one end of the family than at the other, regardless of whether the family be large or small.

One female may lay both fertilized eggs and male eggs.

DISCUSSION

The first stage in the solution of the problem undertaken in these studies seems to have been reached in Experiments IX and X. The results of these experiments indicate that in Hydatina senta the proportion of male-producers is reduced by certain dissolved substances in the water in which the rotifers are reared. The full force of this discovery is not at first apparent, and the explanation of the two experiments in question is not the measure of its importance. Not only may nearly all the results of experiments dealing with the proportion of male-producers, which are described in this paper, be accounted for by this new factor; but practically all the work of previous investigators, which led to contradictory conclusions, may be simply explained by the same means. It is thus possible, without wholly rejecting the conclusions of earlier workers, to bring their apparently discordant results under a common point of view.

In starvation experiments, it is not practicable to use a smaller quantity of protozoan food, without at the same time introducing a smaller quantity of the substances dissolved in the food culture. The results attributed to starvation may in reality be dependent on the reduced quantity of such substances. The difference in the proportion of male-producers apparently resulting from starvation is of the same sign as should result (according to Experiments IX and X) from the influence of these dissolved substances; and as the differences obtained in the starvation experiments (III and IV) are not greater than may easily be explained by this factor alone, it may be doubted whether starvation *per se* has any effect whatever. Nussbaum's conclusion that starved rotifers yielded more male-producers than well-fed ones, seems at first sight to be justified; but the effects which he noted were probably due, not to the scarcity of protozoan food, but to less concentration of certain substances in the water.

A similar explanation is at hand for the "sex strains" described by Punnett. The various series of generations in which this investigator found constant differences in the proportion of male-producers were probably reared on different food. Though the dates of the experiments are not given, it seems almost certain, from the author's account of them, that they were performed at different times, hence different food must have been used. It is thus possible that the constant differences which Punnett noticed were due to constant differences in the nature and quantity of the substances contained in the food cultures. It is conceivable that "strains" may occur in the sense that families derived from rotifers having very different histories may behave differently with respect to the proportion of male-producers. Work is needed to decide this point. But Punnett's explanation does not apply to series of families derived from sister individuals, and recourse to it is not necessary in other cases if the food cultures are different.

The influence of the character of the food cultures is again shown in the experiments (V and VI) with old and new food. The rotifers fed from the old cultures include fewer male-producers than do those given fresh food. As the protozoa in the two cultures were apparently equal in all respects, the cause of the difference in the proportion of male-producers must be sought in the liquid portion of the culture. If it be supposed that the substances in the cultures, which tend to reduce the number of male-producers, accumulate with increasing age of the culture, the smaller proportion of male-producers in families fed from old food is placed in harmony with the other experiments.

Such an accumulation of substances in old food cultures may account for other phenomena. The experiments (XI and XII) in breeding from the first and last members of the family may, on this assumption, be brought into harmony with the general conclusion. At the time when the first daughter in a family was hatched, the food culture from which her mother was fed was three to five days old; when the last daughter was hatched, the same food culture was eight to eleven days old. The food culture had grown older in the dishes with the rotifers, just as it had

in the culture jars, and had probably, notwithstanding its diluted state, gone on accumulating the same dissolved substances. This aging of the food culture is probably not sufficient to account for the very large differences obtained in some of the experiments; but in addition to this factor, there is the possibility that the products of metabolism of the rotifers themselves have the same effect as the substances derived from the food cultures. These two factors may account for the result of the four experiments in which there is a markedly higher proportion of male-producers among the first-born. If, in the experiments, it became necessary to change the water in one of the dishes shortly before the end of the family, and hence to add new food, the last daughter would be hatched under approximately the same conditions as the first. In such a case the result might be like that of the second part of Experiment XII, a nearly equal proportion of male-producers in both first- and last-born. Unfortunately, when these experiments were performed, I did not greatly suspect the influence of dissolved substances in the water. I have no notes, therefore, to show whether or not the water and food were changed as I have suggested. I only know that such changes were occasionally made, but do not know where.

The conclusion that substances in the water cause the variation in the percentage of male-producers is quite in harmony with the nearly uniform results of the experiments (VII and VIII) with drainage water; and since food cultures must be frequently changed, fluctuation in a long series of generations, like that in Experiment I, is probably due to the same cause. Thus practically the whole range of phenomena so far noted, which relate to the variable proportion of male-producers *may* be dependent on this one factor. Some of the explanations I have offered must be provisional only. I am prepared to find that several factors are at work instead of one; but the simplicity of the explantion in every case has led me to extend it tentatively to several phenomena where its validity can be established only by further work.

So far in this discussion, nothing has been said regarding temperature, the factor to which Maupas attributed the most extraordinary differences in the proportion of male-producers. Some

experiments of my own which, because they are too few to be conclusive and because other experiments along the same lines are still in progress, are reserved for a future paper, seem to indicate that temperature has some influence. But as it is probable that the details of Maupas's conclusion can not stand, I have been led to seek for an explanation of his results. Whitney, it will be remembered, has already offered two possible explanations. First, he discovered that at high temperatures a male-producer laid two to four times as many eggs as did female-producers at the same temperature. Maupas probably supposed that the out-put of eggs was the same for each, hence his 97 per cent was in part accounted for, Whitney believes, by larger families. This explanation, however, would only account for an excess of males, whereas Maupas obtained an excess of male-producers. It made no difference in Maupas's experiments whether a male-producer laid 15 eggs or 50, she counted only one toward the 97 per cent in the result. To sustain Whitney's point it would be necessary to show that a female whose offspring are largely male-producers lays more eggs at a high temperature than does a female whose offspring are largely female-producers. Such evidence is not, I believe, forthcoming.

The second explanation offered by Whitney to account for Maupas's results was that at a high temperature shorter families were produced than at a low temperature. He found from an examination of 23 families that the male-producers occurred chiefly in the first two-thirds of their respective families. If these families were shortened by cutting off the last members, which were nearly all female-producers, the percentage of male-producers would be increased. It appears, however, from the examination of a very much larger number of families (Table XV) that the male-producers are not accumulated at either end of the family. It also appears that a short family is not a long family minus its last portion. Families containing 46 to 50 members have their maximum number of male producers among the 25th to 30th members. If a family of 31 to 35 were the same as a family of 46 to 50 with its last 15 members omitted, the maximum number

of male-producers should here also appear among the 25th to 30th members. But it does not; the maximum is among the 15th to 20th members. The same fact emerges from a comparison of any other two groups in Table XV. A short family is not a curtailed long family; it is built on a plan of its own, which is approximately the same, relative to its length, as that of a large family. Either the family is completely worked over, or elimination occurs all along the line, from beginning to end of the family, and not at the end alone.

Since in the light of new data these two proposed explanations are inadequate, I have been led to seek for others. Firstly, Maupas may have used different food for the two parts of his experiments, but his account is too brief to enable us to judge on this point. Another possible, and I believe more plausible, explanation is found in the effect of breeding from different parts of the family. It appears that breeding from the first member of successive families may yield many more male-producers than does breeding from the last member. When the conditions are such as to produce many male-producers among the first-born, the difference may be very great,—57 per cent and 14 per cent respectively in one case. Whether this phenomenon is due to aging of the food culture in the dish with the parent, or to accumulated metabolic products of the rotifers themselves, or to any other factor, does not concern us here. The fact remains that the first-born may yield more male-producers than the last-born. If Maupas reared the first five members of the family at a temperature of 26° to 28° C., and only decided to institute a control experiment when it became apparent that the first families would be largely male-producers, then the sister individuals used for the control and placed at a temperature of 14° to 15° C., must have been late members of the family. Maupas does not tell us that these experiments were performed simultaneously, and it would have been very natural to have tested the high temperature to see whether it offered any probable results before beginning any formal experiments. I offer this explanation only as a suggestion, but it seems to me a probable one.

Since all the positive results upon which earlier conclusions were based may readily be explained as due to substances in the water, let us see whether the negative results offer any obstacles. There is but one point of any considerable importance on which my results are seemingly at variance with those of previous workers. This relates to the question of starvation. The experiments of Punnett and Whitney went to show that quantity of food, or any concomitant factor, had no influence upon the proportion of male-producers. Though I have arrived at an opposite conclusion, my results are not, it seems to me, opposed to their results. Starvation in the experiments of Punnett and Whitney was limited to a period of hours after hatching, whereas in mine it continued throughout life. It was supposed that the female would be more susceptible early in life than afterwards. If the sex of the immediate offspring of a female were to be affected, probably only those factors which operated early in life would be of any avail. If the effect of (apparent) starvation is not noticeable until the second generation, there may be late stages in the development of oogonia and eggs which are more susceptible to the influence of starvation (really the small amount of certain substances in the water) than are the very early stages shortly after hatching. In Nussbaum's experiments and in mine, this influence occurred in late stages as well as early, and it is impossible to state whether the critical period, if such exist, occurs at one stage or at another. Whether my starvation experiments differ essentially, therefore, from those of Punnett and Whitney, seems to depend on whether the influence of the substances dissolved in the water is felt in the first generation or the second. Both Maupas and Nussbaum discussed this point with regard to certain external conditions, but disagreed in their conclusions. Some light is thrown on this question by the experiments with the filtrate from old food cultures. Among the females transferred from spring water to the filtrate, some were male-producers; but of the females of the next generation, none were male-producers. This shows that the full effect of the filtrate is not apparent until the second generation. Whether the proportion of male-producers among the females transferred to the filtrate

was altered by the change of medium, the numbers used were too small to decide; but the influence felt in the first generation is at most only a fraction (probably a small one) of that apparent in the second generation. Since this is the case, it is readily seen how a reduction in the quantity of substance in the water at a comparatively late stage of the rotifer's life, when the oögonia or eggs are well developed, might result in more male-producers in the next generation, whereas a similar reduction just after hatching might have no effect. This view harmonizes with the result that, while starvation throughout life is followed by an increase in the proportion of male-producers, starvation for only a short period after hatching has no effect.

There remains the further possibility that starvation just after hatching may defeat its own purpose. Doubtless some unassimilated food comes over from the egg to the young female. How long this lasts has not been determined. Since a young female eats very soon after hatching, to deprive her of food must disturb her normal processes; but it seems to me doubtful whether as great a degree of starvation has thereby been obtained as has been supposed.

Not less important than the relation of my experiments to those of previous workers, is the examination of the experiments for defective points. The assumption that male-producers appear chiefly in a given part of the family, and that reducing the size of the family may be accomplished by omitting, unaltered, some specific portion of the family, have been shown to be without foundation. As a corollary of this, the similarity of large and small families with respect to the relative position of the male-producers in them shows that in general the value of experiments with Hydatina is not diminished because the families are small, provided the requisite aggregate number of individuals is obtained.

Death losses sometimes invalidate experiments, and must always be taken into account. If any safe conclusion is to be reached, the differences caused by the conditions of the experiment must be so great as to make the death losses insignificant, or it must be shown that these losses are not selective. In the experiments with the filtrate from old food cultures, it has been

shown that the death losses are probably not selective; and even if selective they are, in certain experiments, entirely insignificant. Nothing has been said on this point regarding the starvation experiments. If it could be shown that the shortening of the families in these experiments were due to death of many of the female-producers, a considerable increase in the proportion of male-producers would be accounted for, and the experiments might only show that when the rotifers were starved many female-producers died. That the starved families were smaller was due chiefly to the fact that fewer eggs were laid, and only in small part to failure of the eggs to hatch or to death of the young rotifers. But no amount of elimination of female-producers could result in an increase in the *absolute number* of male-producers. Such an increase in the number (as well as proportion) of male-producers is found in the second and sixth parts of Table IV, an increase too great to be insignificant. I conclude, therefore, that death losses do not vitiate the results of the starvation experiments.

Previous workers with Hydatina have spoken of the problem which its varying proportion of male-producers presents, as one of sex-determination; but it is open, as I have indicated in the introduction, to interpretation as a change from the parthenogenetic to the sexual phase of the life cycle. This view was adopted by Morgan ('07, p. 346). The assumption necessary to support this view was that the male-producers are the sexual females, which assumption was based on the numerical relations found by Maupas ('90b), the cytological evidence of Whitney ('09), and the analogy afforded by Asplanchna (Lauterborn, '98, p. 178). Since among many thousands of females laying only eggs that develop parthenogenetically, not one has ever been found to produce offspring of both sexes, my observation that a male-producer may also lay resting eggs, though not a complete demonstration, leaves little doubt that male eggs and sexual eggs are identical. My observation does not exclude the possibility that female eggs may also be fertilized, but Maupas's experiment mentioned in the introduction and the chromosome counts made by Whitney make this improbable.

If resting eggs are fertilized male eggs, and never fertilized female eggs, male-producers are the sexual females. The appearance of male-producers then becomes merely a transition from the parthenogenetic to the sexual phase of the life cycle, and is not different from that in certain aphids. In aphids it has been shown (Slingerland, '93, and others) that external conditions may influence the occurence of the sexual generation, and Issakówitsch ('07) and Woltereck ('09) find the same true of daphnians. There is a much larger body of earlier literature upon the subject, but any discussion of this, or any attempt to relate the phenomena in the rotifers to those in other groups, is purposely deferred until my data are more complete. In cases where the sexual female is distinguishable from the parthenogenetic female, there has been no confusion of the phenomena of the transition from one phase to the other with sex-determination. Where, as in Hydatina, the sexual is not externally distinguishable from the parthenogenetic female, the inauguration of the sexual phase appears to be merely the addition of males, hence the application of the term "sex-determination" to the phenomena. The external similarity of the two kinds of females does not alter the essential nature of the case. Under this view, the interesting features of the life cycle of Hydatina senta are that the sexual eggs may develop without fertilization, in which case they produce males, and that the two sexes of the sexual phase do not first appear simultaneously. The sexual female always appears one generation earlier than the male, for she is, if unfertilized, the mother of the males.

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THE MECHANISM OF MEMBRANE FORMATION AND OTHER EARLY CHANGES IN DEVELOPING SEA-URCHINS' EGGS AS BEARING ON THE PROBLEM OF ARTIFICIAL PARTHENOGENESIS

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With Two Figures

The first visible change occurring in many eggs after the entrance of a spermatozoön is the appearance, at the periphery of the egg, of a fertilization membrane. Although observed and discussed by many authors, very little experimental work has been done on the mechanism of its formation. Yet such an investigation may give a clue to the nature of the change initiating development.

This summer (1909) a study of membrane formation in Echinoderm eggs was undertaken. The experimental work was performed in part at the Biological Laboratory of the Carnegie Institution at Tortugas, Florida, and in part at the Marine Biological Laboratory at Woods Hole, Massachusetts. I wish to express my thanks to Dr. Ralph Lillie for the use of some reagents and to the Wistar Institute of Anatomy for a table at the latter station. I am also indebted to Dr. T. H. Morgan for very kindly criticising this paper.

The forms experimented on at Tortugas were Toxopneustes variegatus and to a less extent Hipponoë esculenta. At Woods Hole Arbacia punctulata was used.

I shall discuss the early changes taking place in developing eggs under seven heads, viz:

1 The efficiency of acetic acid in forming membranes at different temperatures.

2 The mechanism of membrane[1] formation.

[1] Unless otherwise stated, by membrane, the fertilization or vitelline membrane is meant. The surface film or plasma membrane of the egg is spoken of as the egg membrane. The two have very different properties both chemical and physical.

3 The chemical nature of the membrane.
4 The migration of the pigment granules of Arbacia eggs.
5 Loss of pigment in Arbacia eggs.
6 Surface tension changes in fertilized and unfertilized eggs.
7 The action of development-starting substances in general.

I. THE EFFICIENCY OF ACETIC ACID IN FORMING MEMBRANES AT DIFFERENT TEMPERATURES

A well known method of determining whether a given process occurring in organisms is chemical or physical in nature, is to compare its temperature coefficient with the temperature coefficients of various known physical or chemical phenomena. In this way it has been shown that the rates of increase of the heart beat, conduction of the nerve impulse and many other organic processes are due to chemical processes since they are accelerated to the same degree by a rise of temperature as is the velocity of chemical reaction. The latter are distinguished from the great majority of physical processes, in that they are affected enormously by a rise in temperature. Chemical reactions proceed two to three times more rapidly with every 10° rise in temperature. The same method may be used to see whether the action of a given substance is chemical or physical in nature.

My object in studying the effectiveness of acetic acid at different temperatures was primarily to test Loeb's hypothesis, that the reason the fatty acids are the most efficient acids in calling forth membrane formation is because of their property of dissolving lecithin and other lipoids. Solution is a physical process. The solubilities of most substances are not greatly affected by temperature. Unfortunately the exceptions to the above rule are mostly exhibited by fatty substances. As nothing is known of the solubility of lecithin in acetic acid at different temperatures, a definite answer as to the action of acetic acid on the eggs cannot be given. Certain other possible actions, however, are excluded and certain others included by my results. These will be discussed after giving the results.

The experiments were performed on the eggs of Toxopneutes variegatus. Perfectly normal membranes may be produced on returning to sea-water after the acid treatment. With Hipponoë the membranes formed after treatment with acetic acid are very close to the egg and almost invisible with the low power, except on slightly high focus when they appear separated from the egg surface by a very fine clear ring. This is not apparent in the untreated eggs, and in those eggs which have not responded to the treatment.

In all experiments the usual precautions against contamination with sperm were taken. About 2 cc. of sea-water, densely crowded with eggs, was pipetted to 100 cc. of the acid sea-water at the proper temperature. The eggs were then removed from the solutions at intervals to sea-water at 29° C. (the normal summer temperature of the water at Tortugas) and examined for membrane formation. The temperatures given were readings taken at the beginning and end of the experiment. Controls were always kept.

EXPERIMENT I

June 30, 1909. Eggs taken 4.45 p.m.
2½ cc. $\frac{N}{10}$ acetic acid to 50 cc. sea-water.

TEMPERATURE	TIME IN MINUTES AFTER 5.05 P. M.							
	¼	½	1	2	3	4	6	8
16°-19° C.....	none	none	very few	50%	50%	75%	100%	100%
26°-28° C.....	none	none	occasional	very few	none	65%	10%	none
34°-36° C.....	occasional	50%	50%	10%	occasional	none	none	none

EXPERIMENT II

July 1, 1909. Eggs taken 7.30 a.m.
3 cc. $\frac{N}{10}$ CH_3COOH to 50 cc. sea-water.

TEMPERATURE	TIME IN MINUTES AFTER 8.15 A. M.								
	¼	½	1	1½	2	3	4	6	8
11°-16° C.....	none	none	none		occasional	occasional	50%	a few	a few
19°-20° C.....	none	none	occasional		30%	70%	30%	a few	a few
29°-30° C	50%	70%	90%	100%	90%	40%	none	none	none

EXPERIMENT III

Temperature 23°-24°C.

TIME IN MINUTES AFTER 1.20 P. M.	cc. $\frac{N}{10}$ ACETIC TO 50 cc. S. W.					
	1 cc.	1½	2	3	4	6
1½ min	none	none	25%	45%	50%	80%
3 min	occasional	occasional	70%	*	90%	60%
6 min	occasional	10%	90%	90%	90%	none

Temperature 34°-33°C.

1½ min	none	10%	100%	80%	none	none
3 min	occasional	100%	90%	none	none	none
6 min	20%	100%	none	none	none	none

*Missed.

The above tables may be simplified as follows:

EXPERIMENT I

Optimum time of exposure

Temperature	*Minutes*
18°	7
28°	4
35°	¾

EXPERIMENT II

Optimum time of exposure

Temperature	*Minutes*
14°	4
19°	3
30°	1½

There is about a halving of the time of exposure required for a rise of 10° C. When the optimum concentrations instead of the optimum times are compared as in Experiment III the following result is obtained:

TIME OF EXPOSURE	TEMPERATURE	OPTIMUM CONC. OF ACID
1½ minutes	23°	6 cc. $\frac{N}{10}$ acid to 50 cc. s. w.
	33°	2–3 cc. $\frac{N}{10}$ acid to 50 cc. s. w.
3 minutes	23°	3–4 cc. $\frac{N}{10}$ acid to 50 cc. s. w.
	33°	1½–2cc. $\frac{N}{10}$ acid to 50 cc. s. w.
6 minutes	23°	3 cc. $\frac{N}{10}$ acid to 50 cc. s. w.
	33°	1½ cc. $\frac{N}{10}$ acid to 50 cc. s. w.

Both the optimum-time and optimum-concentration figures show a large increase in the efficiency of acetic acid with a rise of temperature of 10° C. Expressed in terms of a temperature-coefficient $(Q)_{10}$ the increase amounts to a doubling, thus:

$$Q_{10} = \frac{Kt}{K_{t+10}} = 2$$

in which Q_{10} is the ratio of a constant at a temperature t degrees, to a constant at t + 10° C. The very marked efficiency at 36° may be an additive effect as high temperatures are known to cause membrane formation in both sea-urchins and starfish eggs. I did not succeed in producing membranes on Toxopneustes eggs by exposure to sea-water at those temperatures and for the times used with the acid treatment.

What changes might the dilute acetic acid bring about in the egg of a sea-urchin which would result in the formation of a membrane? The chief possibilities are three. It might:

1 Dissolve out the lipoids at the periphery.

2 Change the surface tension of the egg *directly*, in that the composition of the medium about the egg is altered. Instead of egg protoplasm—sea-water, we have egg protoplasm—acid sea-water.

3 Combine with some of the egg proteids.

The first possibility has already been discussed.

The surface tension between two phases is only very slightly influenced by temperature. For the same reason diffusion rate and degree of dissociation of CH_3COOH would be negligible factors.

There remains only the most probable action, an actual combination of the acid with some of the egg proteids, the rate of formation of this compound varying with the temperature as do other reactions ($Q_{10} = 2-3$).

Greeley's[2] results with HCl also bear out this conclusion

Assuming that the acetic acid actually takes part in some reaction[3] in the egg ,which is it? Is the membrane—for there is now ample evidence (to be discussed below) to show that the membrane is not present before fertilization—a result of the union of CH_3COOH with some egg substance? It can be definitely said that this is not the case. The many substances which will produce membranes are so diverse, chemically, that it is inconceivable they should all combine to form the same substance (membrane) or even by their presence bring about its formation. It is obvious that heat and mechanical agitation could not act in this way. The membrane is all ready to be formed yet is prevented from so doing by something. It forms only another example of the so-called "stimulus reactions," which have been compared to the setting off of a charge of gunpowder by a spark. The change which "sets off" the membrane formation as well as the reactions into which I believe the acetic acid enters will be discussed in the second division of this paper.

II. MECHANISM OF MEMBRANE FORMATION

I shall first propose an explanation, of how a membrane may be conceived to form about a system of interacting substances, (as an egg), and then discuss somewhat more fully various facts connected with its actual production.

As observed in the living egg, almost immediately ($1\frac{1}{2}$ to 3 minutes) after the addition of sperm the membrane substance becomes separated from the egg surface by spaces. These spaces fill with a fluid, unite and enlarge, thus pushing out the membrane

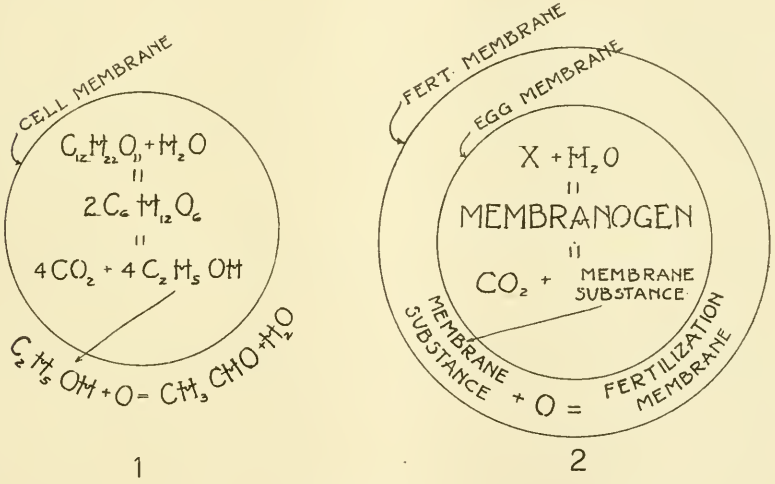
[2] Greeley: A. W. Biol. Bull., iv, 1902-3, p. 124. Greeley did not interpret his results with reference to chemical action.

[3] Reaction is used throughout this paper in the same sense as in chemistry.

some little distance. Two separate events take place, the formation of the membrane, and its separation from the egg.

Mechanism

In order to simplify conditions as much as possible let us consider what would occur under certain conditions in an egg cell (Fig. 2) in which everything has been removed except those substances directly connected with the membrane reaction. Its



Figs 1 and 2.

inorganic analogue would be represented by an hypothetical cell (Fig. 1) containing cane sugar and appropriate enzymes.

The ractions indicated above will proceed until all the substances are present in definite proportions. Equilibrium is then attained. The egg (cell) membrane is impermeable to the contained substances and also to the salts of sea-water. This represents the condition in the mature sea urchin egg. Suppose now a *momentary* change of permeability occurs so that CO_2 + membrane substance (CO_2 + C_2H_5OH) may pass out of the system. This upsets the equilibrium and the reactions proceed in the direc-

tion of the arrows until checked by a second accumulation of reaction products and equilibrium is again attained.

The membrane substance, in contact with sea-water, hardens, (presumably an oxidation and comparable to the hardening of silk in the air) thus forming a film. Some proteid substance formed just behind the fertilization membrane (possibly a small amount of the membranogen diffuses out during increased permeability) would absorb sea-water and push the membrane out.

A second increase in permeability would result in a repetition of the process with the formation of a second membrane.

The principle of Gibbs as applied by Metcalf[4] may help in understanding how this reaction can proceed so readily at the cell boundary. If, in a solution, a reaction occurs, one of the products of which lowers the surface tension of the mixture, the commencing of the reaction will be favored at the surface and the products will collect at the surface.

The rôle of the acetic acid in membrane formation would be the increasing of the permeability of the egg membrane. This is presumably brought about by a combination of CH_3COOH with some of the surface proteids, a change with which increased permeability is assumed to be connected. At the end of this paper I shall give some further general evidence for the permeability theory.[5]

The actual process of membrane formation as observed under the microscope reveals nothing contrary to the above theory. Herbst[6] in 1893 cut sections of eggs, fixed at intervals from immediately after fertilization till the pushing out of the membrane. He describes the clear "Protoplasmasaum" becoming plainly thicker just before a portion of it becomes lifted off and he interpreted this to indicate a secretion. It is not very resistant first but later becomes quite firm. The pushing out from the

[4] Metcalf: Zeit. Physic. Chem. 52 p., 1905; also Höber, Physikalische Chemie d. Zelle und Gewebe, 2 ed. Leipzig, 1906, p. 209.

[5] See my preliminary report (Year-book, Carnegie Inst., Washington, no. 8, pp. 119, 1909), and Science, n. s., xxx, p. 776, 1909, also similar evidence by Lillie, R. S. (Biol. Bull., xvii, p. 202, 1909). and McClendon (Science, n. s., xxx, p. 454, 1909).

[6] Biol. Centralb., 13, 1893, p. 14.

egg is due to "eine gallertartige Substanz, welche durch von aussen aufgenommenes Wasser aufquillt." Loeb[7] has recently expressed the opinion that an "Eiweisskörper" or lipoid is the substance concerned in the absorption of sea-water and resultant separation of the membrane.

I had come to similar conclusion this summer before having read Loeb's or Herbst's papers. The facts are as follows: The fluid between the egg and fertilization membrane has too great a volume to have come from the egg without a corresponding diminution in size. It must be chiefly sea-water. It at least contains considerable chlorides (as shown by precipitation with $AgNO^3$). The fertilization membrane is very freely permeable to the salts of sea-water, relatively impermeable to sugar and proteids. A small concentration of a sugar or proteid (even though its osmotic pressure were far less than that of sea-water) would be capab'e of absorbing sea-water through a membrane perfectly permeably to sea-water.

Double Membranes

I have already mentioned the result we should expect in a hypothetical sugar cell if the permeability of its membrane should be a second time momentarily increased, namely, a second escape of the reaction product. In the egg a second membrane should be formed by substances causing a second increase of permeability. This actually occurs. Tennent[8] has recorded a second membrane formed by sperm on starfish eggs which had previously been treated with CO_2 and formed membranes. I was unable to get the above result in sea-urchin (Toxopneustes) eggs treated with CH^3COOH followed by the addition of sperm. Only those eggs which had been subjected to the acid treatment too short a t'me to form membranes could be fertilized. They segmented normally. The spermatozoa were apparently unable to pass membranes formed by acid.

[7] Loeb, J.: Arch. Entwm., xxvi, 1908, p. 82.

[8] Tennent: Journ. Exp. Zoöl., 31, 1906, p. 538.

Chloroform saturated sea-water causes membrane formation in a large per cent of Toxopneustes eggs. If the eggs have been fertilized first, beautiful examples of double membranes can be obtained by after treatment with $CHCl_3$ saturated sea-water.[9] The second membrane (due to $CHCl_3$) is just as distinct as the true fertilization membrane and lies half way between it and the egg surface. These eggs are also more normal looking than unfertilized eggs subjected to $CHCl_3$ sea-water treatment as the cytolytic changes caused by the $CHCl_3$ do not take place so rapidly on eggs with membranes already formed, probably because the membrane offers some resistance to its ready entrance. This second membrane can be formed on eggs which are in the two cell stage and also up to early blastulæ.

In the two cell stage it is as distinct as in undivided eggs and surround each of the blastomeres. The greater the number of blastomeres the less distinct does it become and the more closely does it surround each cell. The space between it and the cell surface also becomes less transparent as if filled with some other constituents of the blastomeres which have diffused out. The fact that membranes can be produced so late in segmentation stages indicates a considerable similarity in the constitution of the egg during early cleavage stages.[10]

An extremely fine and delicate second membrane is formed on fertilized Arbacia eggs placed in four times concentrated sea-water as also on the evaporation of the sea-water on a slide. This is accompanied by loss of pigment and other constituents of the eggs.

Attempts to produce secondary membranes in fertilized Toxopneustes eggs with CH_3COOH failed, even though they were treated long enough to bring about the characteristic effects of over treatment.

[9] Herbst had already performed this experiment. Loc. cit.

[10] On examining the literature I find that Loeb (Arch. Entwm. 23, 1907, p. 479) has already recorded membranes formed on individual blastomeres. In one case 2-4 cell stages were obtained by $CaCl_2$ treatment (50 cc. $\frac{2}{3}$ *m* $CaCl_2$ + 1.6 cc. $\frac{N}{10}$ NaOH). In another case 2-16 cells were produced by hypertonic treatment. In both cases when sperm was added the individual blastomeres became entirely surrounded by a membrane and dwarf gastrulae resulted from their further development.

Different Types of Membranes

Loeb[11] has cited several instances of development without membrane formation in sea urchins and R. Lillie.[12] has mentioned such a case in starfish. The best known case is presented by development after treatment with hypertonic sea-water. I have repeated this experiment of Loeb's (using 100 cc. sea-water + 15 cc. $2\frac{1}{2}$ *m* KCl for 1 hour 20 minutes) and find that there are membranes formed on these eggs exactly like those formed on Hipponoe eggs treated with CH_3COOH already mentioned. They are very close fitting and might easily escape notice. Membranes which push out only very slightly from the egg surface may be produced by sperm fertilization at high and at low temperatures (15° to 20° C. with Toxopneustes at Tortugas, and 32° C. with Arbacia at Woods Hole). The eggs are mixed with the sperm for about one-half minute and then placed in the sea-water at the proper temperature. Cases of development without membrane formation seem to be rather cases of development without pushing out of the membrane.

Another type of membrane is obtained by allowing eggs to stand at room temperature for 28 hours. When sperm is added practically all the eggs become surrounded by a thick membrane adhering to the egg surface closely. When the egg divides this surrounds each of the blastomeres, which become quite spherical. Similar membranes are formed by sperm fertilized eggs in Ca-free sea-water.

That the fertilization membrane is not present as a surface film in unfertilized eggs which is later pushed out is shown by the fact that unfertilized eggs dissolve completely in concentrated H_2SO_4 while fertilized eggs dissolve all but the membranes.

[11] Loeb. J. (1) On fertilizing with sperm after 48 hours standing in sterilized sea-water. Pflügers Archiv. 93, p. 59, 1903. (2) By hypertonic sea-water. Univ. Calif. Pub. Phys., ii. p. 83, 1905. (3) After treatment with pig serum some eggs form no membranes. These may segment and develop into larvae. Arch. f. d. ges. Physiol. 124, p. 250, 1908. (4) Some eggs of Asterina form no membranes after acid treatment, yet develop into small blastulæ, Univ. Calif. Pub. Phys. ii. p. 153, 1905.

[12] R. Lillie. After 20 hours in $\frac{M}{2000}$ KCN eggs are warmed. No membranes form yet segmentation takes place. Journ. Exp. Zoöl. v, p. 386, 1908.

III. CHEMICAL NATURE OF THE MEMBRANE

Experiments were also undertaken to determine the composition of the fertilization membrane. Arbacia eggs were used.

Their membranes are insoluble in *m*KOH and NaOH even on short boiling, although the eggs become entirely colorless only a few granules being visible. On prolonged boiling and evaporation when the strength of the alkali must approach $2\frac{1}{2}$ *m*, the membranes dissolve or at least become so broken up as to be invisible.

In cold concentrated H_2SO_4, the membrane is insoluble while the egg substance first chars reddish brown, later becoming entirely invisible so that only the spherical fertilization membrane is apparent. Unfertilized eggs without membranes dissolve entirely in concentrated H_2SO_4.

In concentrated HCl there is no solution of the membrane. The egg contents become a clear shrunken granular mass. Eggs in the two cell stage show the division between the blastomeres as a clear line. Concentrated HNO_3, NH_4OH and glacial acetic acid act like HCl.

I was unable to demonstrate any proteid in the membrane by the xanthoproteic test although this may be due to its thinness. At any rate the membrane appeared colorless while the egg contents were turned a bright yellow.

Lillie[13] has recently expressed the opinion that the fertilization membrane is "a paptogen membrane consisting mainly of protein material." Such a membrane would be much more delicate and easily ruptured than the fertilization membrane is. Besides there appears to be little if any protein in it as shown by its insolubility in pepsin HCl, caustic alkalies and concentrated H_2SO_4. It may be compared to the cellulose layers formed about plant cells after division, or to the chitinous skeleton formed in insects by the hypodermal cells. In composition it is probably one of the albuminoids.

[13] Lillie, R.: Biol. Bull., xvii, pp. 202, 1909.

SUMMARY

The essential points brought out in the preceding pages may be summarized as follows:

The action of acids in producing membranes on unfertilized sea-urchin eggs is due to their combination with some substance in the egg but the membrane is not the product of this combination.

In composition the membrane is probably an albuminoid. It is not present as such before fertilization.

The essential condition for its formation is an increased permeability of the egg surface for a membrane substance which passes out and hardens to the membrane in contact with sea-water (a secretion). Double membranes may be explained on the above theory.

Several types of membrane may be produced under different conditions and it is probable that the secretion of the membrane substance always takes place although it may remain close to the egg surface.

IV. MIGRATION OF THE PIGMENT GRANULES OF ARBACIA EGGS

The second visible change which takes place after fertilization in Arbacia eggs is the migration of the red pigment granules to the surface. In the mature unfertilized and the immature eggs they lie distributed throughout the cytoplasm. This change takes place within ten minutes after fertilization and invariably whenever membrane formation takes place, no matter by what means brought about, whether by acid, by osmotic treatment or by sperm. It is thus associated with membrane formation and may be explained as follows: Most small particles suspended in fluid media become negatively charged and there is additional evidence that these pigment containing bodies are so charged. Lillie[14] has brought together evidence that the centrosomes are

[14] R. Lillie: Am. Journ. Physiol., xv, p. 46, 1905.

negative regions. When the micromeres are formed the pigment is prevented from entering them by the large and prominent asters, then present. Even when cut off from the pigmented area of centrifuged eggs these cells are relatively free of pigment.[15] The granules are thus repelled by the centrosomes. If an increase of permeability is the change initiating the development of an unfertilized egg the same potential differences (between exterior and interior, and different regions of the cell) might be expected that takes place in muscles during stimulation. These potential differences are quite general in the functioning of various tissues (nerves, glands, sensitive plants, etc). Their origin is most easily accounted for by variations in differential permeability of the cell to anions and cations.[16] Lillie[17] has discussed this theoretically in a recent paper. Without going into details it may be said that "with the appearance of an increased permeability the peripheral regions of the protoplasm must become, for a time at least until the potentials are equalized, *positive* relative to the interior." Such pigment granules if negatively charged would be drawn by the electrostatic attraction of the now positive egg surface, to the surface, providing of course, the potential difference were high enough. A calculation (by Lillie) of this based on the observed changes in muscle cells has given a value of 14 volts per cm. This would be ample to account for the migration actually observed in Arbacia eggs.

The orientation of small particles with relation to the asters occurs in other eggs. Fischel[18] in staining sea-urchin eggs with intra-vitam dyes noted a migration of particles stained with neutral red, toward the nucleus and aster, the formation of an ellipse about the spindle-figure and a ring about each daughter nucleus where the cell divides, and finally, during the resting stage, even redistribution throughout the cytoplasm. This process is repeated during each cell division.

[15] Lyon, E. P.: Arch. Entwm., 23, p. 67. 1907.

[16] See Bernstein, Arch. f. d. ges. Physiol. 1902, xcii, and Brunings, id. xcviii, and c. 1903.

[17] Lillie, R.S: Biol. Bull. xvii, p. 207-208, 1909.

[18] Fischel, A.: Anat. Hefte, 37, p. 863, 1899, also Arch. Entwm., xxii, p. 526, 1906.

V. LOSS OF PIGMENT IN ARBACIA EGGS

Since the pigment of Arbacia eggs is soluble in water it follows that the membrane of the chromatophore granules must be impermeable to the contained pigment, otherwise it would diffuse through the cytoplasm and out of the eggs (providing the egg membrane were also permeable to it). If the eggs are heated diffusion of the pigment into the sea-water takes place, showing an increase in permeability in both the chromatophore and plasma membranes. I have never noticed a permeability (to pigment) of the former independent of the latter. There is also evidence that the impermeability of the granule membrane is dependent on the plasma membrane. If unfertilized eggs are crushed slightly under a cover glass, part of their contents will flow out into the sea-water and round up. The colored granules which have been pressed out immediately lose their pigment while those within the original fraction still retain it. I do not think this can be due to crushing because the bodies are so small. There is also a difference in the optical properties of the surface newly formed about the extruded protoplasm as compared with the old.

The same phenomenon is observed when eggs are placed in hypotonic sea-water (sea-water one third, distilled water two-thirds. The eggs swell and their surface layer becomes indistinct.

When this occurs the pigment immediately disappears from granules and diffuses throughout the cytoplasm. At the same time in many eggs a thin irregular membrane separates.

A loss of the pigment, which becomes a yellow-red color, occurs in four times concentrated (by evaporation) sea-water[19]. The same solution occurs in CH_3COOH in concentrations greater than those required to produce membranes. This index of determining permeability changes has been used by Lillie[20] working on muscle cells. Pure isotonic solutions of electrolytes which cause

[19] See Loeb's description of this in Strongylocentrotus U. Calif. Pub. Physiol. ii, pp. 73-81, 1905.

[20] Lillie, R.: Am. Journ. Physiol. xxiv, p. 14, 1909, and id. p. 459.

contraction in the muscles, bring about a loss of pigment in the cells of the same organism, Arenicola larvæ. McClendon[21] mentions that the parthenogenetic agents which he has used bring about a loss of pigment in sufficient concentration, and Loeb[22] had already emphasized the cytolytic nature of membrane forming substances although interpreting it in a different way.

VI. SURFACE TENSION CHANGES IN FERTILIZED AND UNFERTILIZED EGGS

In the same paper[23] which was cited in discussing the cause of the movement of the pigment granules of Arbacia eggs to the surface, Lillie has pointed out the relation which should exist between changes of permeability (accompanied by ionic interchange) and the surface tension of the membrane in question. An increase of permeability should be accompanied by an increase in surface tension (in as much as the surface tension of a film is greatest when the potential difference between its two sides is least). This actually does take place in Echinoderm eggs. Eggs from the same females are often somewhat irregular in shape, frequently being elongated, twice as long as wide. Sometimes 40 per cent of Arbacia eggs are in this condition and I have seen practically all the mature eggs of Toxopneustes irregular just after shedding. It is hard to realize that such small bodies can exhibit the shapes they do especially if they are compared with other fluid systems of the same size, as oil droplets, which maintain their spherical form through their high surface tension. If such irregular eggs are fertilized with sperm or treated with CH_3COOH there is an immediate change. They all become spherical, indicating an increase of surface tension. The rounding of eggs on fertilization takes place quite generally. Whether this is actually due to a change in potential difference resulting from increased permeability is not so certain but it is significant that the change occurs just after the entrance of a spermatozoön or after treatment with acid sea-water.

[21] Science, n. s. xxx, p. 454, 1909.

[22] Loeb, J.: Arch. f. d. ges. Physiol. 122, p. 196, 1908.

[23] Lillie, R.: Loc. cit., p. 204.

Sea urchin eggs (Arbacia and Toxopneustes) also round up on standing for some time in sea-water thus indicating an increase in surface tension. They also become fertilizable by foreign sperm on standing[24] (ca. 6 hrs.) or by treatment with an acid or alkali (in concentrations too weak to cause membrane formation.) It appears as if the change undergone by the eggs on standing were in the direction of increased permeability and that the egg must start toward development in order to be fertilized by foreign sperm. Some eggs do undergo division on standing but it is probable that accessory factors are responsible for this.

In order to determine further the nature of the change taking place in mature unfertilized sea-urchin eggs on standing in sea-water, and especially if this change were in the direction of increased permeability, I tried if any less acid were required to cause membrane formation six hours after shedding. At this time the eggs of Toxopneustes become fertilizable by foreign sperm. The following table is a typical result:

July 14, 1909. Eggs taken 12.30 p.m. Temp. 33° to 34°.

	CONCENTRATION—CC. ACID TO 50 CC. SEA-WATER					
	1 cc.	1.5	2	3	4	6
¾ hour after taking						
1½ minutes	none	10%	100%	80%	none	none
3	very few	100%	90%	none	none	none
6 hours after taking						
1½ minutes	none	very few	100%	80%	none	none
3	very few	30%	90%	very few	none	none

Another experiment like the above gave a similar result, the optimum treatment, both as regards time and concentration, about coinciding three-fourths and 5¾ hours after taking the eggs. If any, a very slightly longer treatment appears more favorable. Certainly the eggs require no less acid to cause membranes to form after standing for six hours. In the above experiment the per cent of eggs which could be caused to form membranes was

[24] See Tennent, Biol. Bull. xv, p. 127, 1908.

less in the lot which had stood. This is also the case when eggs are fertilized with their own sperm. I tried this experiment with Hipponoë with the same result.

Apparently the increase in surface tension on standing is not connected with an increase of permeability as I believe the increase after fertilization to be. The increase in surface tension may account for the entering of starfish sperm which could not enter immediately after shedding of the eggs, for Loeb[25] has expressed the opinion that the surface tension of the egg and sperm are the determining factors in the entrance of the spermatozoön. As the sea-urchin egg stands a decreasing per cent of its own sperm becomes capable of entrance while an increasing per cent of foreign sperm may enter.

VII. ACTION OF DEVELOPMENT-STARTING SUBSTANCES IN GENERAL

Throughout this paper a momentary increase in permeability of the egg membranes has been mentioned as the fundamental change underlying membrane formation and the initiation of development. Eggs in which no membranes are formed are excited to development by the same means as eggs which do secrete a membrane, so that this increase of permeability is probably a change occurring after the entrance of a spermatozoön in all eggs. A consideration of the various parthenogenetic agents bears this out.

A classification of the present known means of causing eggs to develop is as follows:

1 Hypertonic solutions (with an OH ion concentration 10^{-6}). Raising the osmotic pressure of the medium by electrolytes or non-electrolytes or evaporated sea-water. The most universal method for Echinoderms, Annelids, Molluscs and Vertebrates.

2 Hypotonic solutions and distilled water (Asterias and Arbacia), Shucking.

3 Mechanical agitation (Annelids and Star-fish).

4 Temperature changes (Echinoderms).

[25] Loeb.: Dynamics of Living Matter, p. 163.

1 Short exposures to high temperatures.
2 Long exposures to low temperatures.[26]

5 Electrical shocks.

1 Charging eggs (Strongylocentrotus) Delage.
2 Induced shocks (Arbacia) McClendon.
3 Constant current (Asterias) (?) Shucking.

6 Chemical reagents.

1 Specific actions—K, Mg; Mn Ni and Co. (?) (Delage)
2 Alkaloids and glucosides (saponin, solanin, pilocarpin, strychnin, quinin, hyocyamin, nicotin.)
3 Tannin and related substances.
4 Fat solvents (ether, chloroform, benzol, alcohol).
5 Bile salts (Na taurocholate and glycocholate).
6 Blood sera (of rabbit, pig, ox, and certain worms).
7 Acids and alkalies.

7 Absence of oxygen (weak CNK and O-free sea-water). [26]

A glance at the above classification will show the general similarity in the means of stimulating muscles and sensitive plans and of exciting unfertilized eggs to develop. They are both stimulus responses, and may be expected to show a common underlying cause conditioning the response. I have discussed this in a preliminary note in Science[27] and quote from it: "A considerable mass of evidence now exists, especially emphasized in recent papers of Ralph Lillie,[28] that stimulation of muscles is effected by a momentary increase in the permeability of the muscle membrane to CO_2 allowing its more ready escape during contraction. CO_2 is the chief end product of the energy-yielding reaction on which contraction depends and its removal from the cell allows the reaction to proceed (during contraction) to a new equilibrium (of rest) when checked by a second accumulation

[26] Absence of oxygen and low temperature as well as hypertonic solutions (in part) seem to act as correcting agents, setting the oxidations in the egg on the right path to proper development, (Loeb), and as such do not come for discussion within the scope of this paper.

[27] Science, n. s., xxx, p. 694, 1909.

[28] Lillie, R. S.: Am. Journ. Physiol., xxii, p. 75, 1908; xxiv, p. 14, 1909; and xxiv, p. 459, 1909.

of CO_2. The increase of permeability on stimulation removes the condition which is preventing the contraction. The movements of sensitive plants can best be explained as due to an increase in permeability of the cell membranes relative to the turgor maintaining substances. The important point is that processes in general brought about by stimulation are connected with changes in permeability." Morgan expressed the situation clearly when he compared the means of causing development to a stimulus.

The best method of determining permeability changes is by the use of pigment containing cells, such as red blood corpuscles. The escape of haemoglobin serves as an indicator of increased permeability. This process of haemolysis occurs frequently in cases of organic poisoning and is manifested in living animals by haemoglobinuria. In the laboratory loss of haemoglobin (from erythrocytes) can be brought about in various ways, by strongly hypertonic as well as by hypotonic solutions. Brahmachari[29] regards the laking in hypotonic media to be due to some other cause than the actual rupture of the corpuscle by absorption of water. High (heat laking at 60° C.) temperatures, condenser discharges, and a great variety of chemical substances also allow the haemoglobin to escape. Of the latter may be mentioned acids (especially fatty acids) and alkalies, glucosides and alkaloids (saponin, solanin, pilocarpin), tannin and related substances, fat solvents (chloroform, ether, alcohol, benzol), the bile salts (Na glycocholate and taurocholate), soaps, haemolysins of foreign blood sera and of animal (cobra, spider, crotalus venom), plant (Amanita and Helvella) and bacterial poisons.[30]

The list given above coincides almost exactly with the list of chemical substances starting development. As yet there have been no experiments on the poisons mentioned but it is highly probable that reptile, fungus, and bacterial poisons will be found as efficient in causing development as Loeb has shown the bile

[29] Brahmachari, U. N.: Biochem. Journ. iv, p. 280, 1909.

[30] For a discussion of means and substances causing haemolysis, see Stewart, G. N. Journ. of Pharmacology and Exp. Therapeutics, i, 1909, p. 49. Heinz, R..: Handbuch der experimentellen Pathologie u. Pharmakologie, Bd. i, p. 392, Jena, 1904.

salts, solanin and saponin to be. The specific ions may well increase the permeability of egg cells, for their action on other tissues is of this nature. In the alkali and alkaline earth metals this has been very clearly brought out by Lillie's work on Arenicola larvae, already mentioned.

It seems probable that the induced increase of permeability brings about the development of an egg for the same reason that the increase on stimulation brings about contraction in muscle cells, namely, by permitting, at the proper time, the escape of some reaction product which is preventing, by its accumulation, the further proceeding of reactions in the egg. This is one very important way in which chemical equilibria may be upset in fluid mixtures surrounded by membranes whose permeability may vary. The reaction product which escapes may be simply CO_2, (as appears to be the case in muscles) or some more complex substance, (as the membrane substance) or both. A discussion of this with practically no experimental data would be useless, however.

The facts which indicate a momentary increase in permeability of the surface membrane, as the first change taking place in the development of an egg, may be summarized as follows:[31]

1 The general similarity in the means of stimulating eggs to divide, and the means of stimulating muscles and sensitive plants. These may be broadly classified as chemical, mechanical, electrical, thermal, and osmotic.

2 The fact that the chemical substances which start parthenogenesis cause in other cells an increase in permeability (haemolysis of red blood corpuscles and loss of pigment in pigment bearing cells).

3 Evidence that stronger concentrations of development starting substances cause loss of pigment in pigmented eggs.

4 That a secretion is the first visible change occurring in many eggs.

5 That a migration of pigment-containing granules to the cell surface in Arbacia eggs is caused by a region of positive change

[31] These are quoted unchanged from my article in Science.

at the surface resulting from ionic interchange accompanying increased permeability after membrane formation.

6 That an increase of surface tension, which must accompany a change of potential at the surface, is quite general in naked eggs after fertilization, as indicated by their rounding up when previously they had been irregular in outline.

THE EFFECTS OF PARASITIC AND OTHER KINDS OF CASTRATION IN INSECTS[1]

WILLIAM MORTON WHEELER

With Eight Figures

I. THE EFFECTS OF STYLOPIZATION IN WASPS AND BEES

The perusal several years ago of a very interesting paper by Pérez ('86) on bees of the genus Andrena infested with Stylops led me to undertake a similar study of our North American wasps of the genus Polistes parasitized by Xenos. I began to collect stylopized P. variatus during the autumns of 1898 and 1899, while I was living in Chicago, but the wasps proved to be too scarce to serve my purpose. During the summer of 1900, however, while I was spending my vacation at Colebrook, in the Litchfield Hills, Connecticut, I noticed many specimens of Polistes metricus Say infested with Xenos (Acroschismus) wheeleri Pierce and I at once began to collect them.[2]

In ten days during the latter part of August I gathered one thousand specimens of the Polistes from flowers of the golden

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University No. 20.

[2] There may be some doubt about the specific names of the host and parasite here mentioned. I have called the wasp P. metricus as this is the name under which it is commonly known and because our extremely variable species of Polistes are in a state of great taxonomic confusion. Miss Enteman, who has studied them very extensively ('04), would probably refer my specimens to P. pallipes Lepeletier, while others would be inclined to regard them as belonging to P. fuscatus Fabricius. Brues ('09) and I had identified the parasite as Xenos peckii Kirby, but Pierce ('08), regards it not only as specifically, but also as generically distinct. He has given it the name wheeleri and placed it in a new genus (Acroschismus) because it has the œdeagus "considerably dilated at the base, arising between two claws," whereas Kirby's species is placed in another new genus, Schistosiphon, because it has the œdeagus "cleft at the apex." The old genus Xenos of Rossi he restricts to the European species (vesparum Rossi and jurinei Saunders). Although these generic distinctions may prove to be valid, I shall use the old name Xenos in the present paper.)

rod (Solidago canadensis) within an area of less than a square mile and noted the sex of each individual and the number, sex and position of the Xenos parasites which had protruded their heads between the gastric sclerites of the wasps. A further study of the form and coloration of the hosts was undertaken in the hope of detecting modifications, like those seen by Pérez in stylopized Andrenæ. My observations, however, gave much less interesting results than those obtained by the French naturalist, and I therefore refrained from publishing them and awaited an opportunity to continue them on additional material. This opportunity, however, has not presented itself, so that I have decided to give my observations for what they are worth, in the hope that they may be amplified by some other more fortunate observer. My preserved Xenos material was turned over partly to Miss Enteman, who published a short paper on the genital ducts of the females ('99), and partly to Mr. C. T. Brues who published a brief account of the embryology of the parasite ('03). The table on the page opposite contains the results of counting the sexes of both host and parasite on the different dates of collecting.

From this table the following conclusions, valid only, of course, for the particular summer and locality in which the insects were collected, may be drawn:

1. Of the total number (1000) of Polistes metricus, 251 or fully 25 per cent were stylopized. This is a high percentage, though as will be shown, it has been exceeded in the statistics of other observers. It may be regarded as too great, first because the parasitized individuals, being more sluggish, would be more easily caught, and second, because my interest in such specimens would lead me to exercise greater care in capturing them. I would say, however, in answer to such objections, that I attempted to collect the wasps at random without noticing whether they bore parasites or not, that a long handled net was used in capturing them, and that the table contains only specimens in which Xenos had already protruded their heads between the gastric segments of the wasps. A number of apparently unifested wasps were dissected and were found to contain larval parasites, so that the actual percentage of parasitism was even greater than that indicated in the table.

Number of Collection	Date	Total Number of Polistes Taken	Male Polistes	Female Polistes	Total Number of Infested Polistes	Number of Males Infested	Number of Females Infested	Total Number of Xenos	Number of Male Xenos	Number of Female Xenos
	August									
1	14	60	4	56	33	0	33	85	71	14
2	16	72	3	69	31	0	31	67	58	9
3	19	31	5	26	14	2	12	55	49	6
4	20	108	5	103	43	3	40	89	73	16
5	21	73	6	67	18	0	18	36	24	12
6	22	143	6	137	12	3	9	19	10	9
7	23	66	15	51	20	2	18	50	36	14
8	24	137	36	101	21	5	16	40	32	8
9	27	167	50	117	29	8	21	55	34	21
10	29	143	7	136	30	2	28	66	56	10
Totals:		1000	137	863	251	25	226	562	443	119
Aver. and per cent		100	13.7	86.3	25.1	2.5	22.6	56.2	44.3	11.9

2. The number of male Polistes increased very suddenly August 23 to 27 and then fell off still more abruptly. Apparently these collections were made at the time of the emergence of the male brood for the particular locality.

3. The greater difference in the ratio of male to female Polistes (1 : 6.3) is to be accounted for partly by this temporary appearance of the males and partly, perhaps, by the fact that this sex is much more wary and therefore more difficult to capture than the females.

4. While the total number of females examined was somewhat more than six times as great as that of the males, the number of females stylopized was fully nine times as great as that of the stylopized males. As the male brood of the wasp appears late in the season this may be due to a partial immunity of this sex from the attacks of the parasites, since Brues ('05) has shown that the triungulin Xenos must enter the wasp larvæ in the spring or early summer (*vide infra*, p. 393.)

5. The table shows that the sexual ratio of the Xenos (3. 7 males to 1 female) was almost the reverse of that of the sexual ratio of the Polistes. That the male parasites should be nearly four times as numerous as the females is easily explained, however, from the fact that the males are so much smaller than the females that more of them can develop to maturity in a single host.

In addition to these more general conclusion, a number of more

Fig. 1. Specimens of Polistes metrica heavily parasitized by Xenos (Acroschismus) wheeleri.

special deductions may be mentioned, based on the daily tables which are too long and complicated to be inserted here:

1. The number of Xenos in a single Polistes varied from 1 to 11. The latter number was taken from only three wasps and these were all females. Ten Xenos were taken from a single individual, also a female, and as a rule the higher numbers, *i.e.*, 5 to 9 were all taken from wasps of this sex, but one male contained 8 of the parasites. In the great majority of infested specimens only one

or two Xenos were present. The table shows that the average number in all the infested wasps was about 2.4. These numbers probably represent the few survivers of an originally much greater number which had lived as larvæ in the individual larval wasps. Brues ('03) took as many as 31 larvæ of X. pallidus of both sexes from a single larva of the Texan P. annularis!

2. Both sexes of the Xenos may occur in the same Polistes, but when the number exceeds 4, the Xenos are all males. In only one case did I find as many as 3 female Xenos in the same host; in all other cases there were only one or two. In 45 of the 251 infested Polistes, or in nearly 18 per cent, Xenos of both sexes occurred. Hence while there is undoubtedly a tendency, as Brues has observed ('03), for the sexes to be the same in the same host, this is so far from being a general rule, that the sex of the parasite cannot be supposed to be determined by its host.

3. When more than one female Xenos is present in the same Polistes, they are of the same size but each is smaller than the females occurring singly in a wasp.

4. When both sexes inhabit the same Polistes the heads of the females protrude between the more posterior segments, whereas the cephalic ends of the male puparia may protrude between any of the segments behind the first. The heads of the females therefore usually appear from under the posterior edges of the fourth or fifth abdominal segments. This is obviously an adaptation to the greater length of the female parasite, which has to lie stretched out in the abdomen of its host and could not protrude its head between the more anterior segments without bending its body. Sometimes both sexes protrude their heads side by side from under the tergite or sternite of the same segment. Sometimes one sex is on the dorsal, the other on the ventral side of the same wasp, but protruding from the same segment.

5. When the female Xenos protrudes its head between two tergites, it lies with its ventral surface uppermost, *i.e.*, its dorso-ventral orientation is the reverse of that of its host: when it protrudes its head between two sternites, it lies with its ventral surface downward, *i.e.*, with the same dorso-ventral orientation as the wasp. This is obviously an adaptation to copulation with the winged

male, for the latter must have to insert its penis along the ventral surface of the head of the female and immediately under the overlapping sternite or tergite of the host.

That several of the conclusions drawn from the table on page 379 cannot have general validity is shown by comparing them with the statistics of other observers. Horne ('72) says that the specimens of Polistes hebræus which he observed in India were "extremely troubled with Stylops (Xenos), every fifth or sixth one taken having a female of one under one of the segments of the abdomen." Theobald ('92) found that among 180 Andrena lapponica taken in England during 1887, 105 or 58 per cent contained Stylops; of 60 bees of the same species, taken in 1888, 54 or 90 per cent were badly stylopized. He believes that the female Andrenæ are more afflicted with the parasites than the males, and he records the number of Stylops found in the 54 bees taken during 1888 as comprising 33 females and 21 males; 2 females each contained 2, 3 males contained 2, 25 females and 18 males 1 each. The corresponding numbers for 40 stylopized specimens of Andrena nigroænea were 3 females each with 3 Stylops, 1 male with 3, 3 females with 2, 5 males with 2, 16 females with 1 and 12 males with 1, making 22 females and 18 males. On the basis of these figures Theobald differs from Perkins ('92), who found the males of various Andrenæ and Halicti more frequently stylopized than the females. This author says that he has seen hundreds of stylopized male Halictus tumulorum, but has never seen a female in this condition. Although Theobald's conclusions agree with my own, his data do not furnish very strong support in favor of his contention, since in A. lapponica the ratio of parasitized males to females is 1 : 1.5 and in A. nigroænea only 1 : 1.2. Skinner ('03) counted 34 stylopized individuals among 140 Polistes texanus, which he found at Pecos, Texas. He says that "most of the Xenos appeared to be females and only 4 males were secured."

The percentage in this case is very similar to that which I found in P. metricus. Brues ('05) has published some statistics on two colonies of the Texan P. annularis infested with Xenos nigrescens Brues and X. pallidus Brues. In these cases the amount of para-

sitization was very great. In one nest there were 86 wasps, 44 or 51 per cent of which contained X. nigrescens. There were from one to seven in each wasp (an average of 2.6 per host), and of the total number of Xenos (94), 91 were males and only 3 females. In the other nest there were 42 wasps, and 36 or more than 85 per cent were stylopized. The total number of the parasites—in this case X. pallidus—was 125 (81 males and 44 females); the highest number in a single wasp being 10, the average per host 3.6.

Fuller consideration must be given to the effects of the stylopids on their hosts. This may properly begin with a résumé of the excellent work of Pérez (1886) who examined stylopized specimens of 47 species of Andrena. The effects produced by Stylops in these bees is so considerable as to render their specific determination difficult. This is not surprising perhaps, when we consider the vast number of closely related species in the genus. All the known specimens of certain "species" (F. Smith's Andrena insolita, separata and victima) have been found to be stylopized, which gives force to Pérez's opinion that these are not true species but merely parasitized individuals of forms that are already known under other specific names. Pérez describes minutely the following modifications as characteristic of stylopized Andrenæ: (1) The abdomen is shortened and swollen and therefore more globular, the shortening being due to an attenuation of the terminal segments. (2) The head is usually smaller than that of normal specimens. (3) The villosity of the abdomen is more abundant, longer and more silky, especially on the terminal segments, and its color is often greatly altered, becoming lighter and more reddish or fulvous. The villosity of the thorax may undergo similar but less pronounced changes. (4) The punctation of the body becomes finer, denser and more superficial in correlation with the pilosity, which arises from the punctures. These changes are common to both sexes and therefore affect specific characters. They give the specimens a peculiar pseudospecific facies. Pérez therefore rightly warns against basing new species of Andrena on stylopized individuals.

The following changes affect the secondary sexual characters: (1) The normal males of the genus Andrena, as in many other

genera of bees, have a greater amount of yellow or white on the face or clypeus or on both than the cospecific females. Stylopization tends to diminish this light color very perceptibly and hence to make the face of the male resemble that of the female. In the female the parasites produce the reverse effect, making the face resemble that of the male. "It is difficult to find a stylopized male of A. labialis, *e.g.*, whose face is normally colored and, on the other hand, it is quite as rare to find a stylopized female of this species having the face entirely black." (2) The normal female Andrena differs from the normal male in the structure of its hind legs, the tibiæ of which are modified for collecting pollen. They are always robust and incrassated and have a brush of long, curved hairs, especially on their internal surfaces. Similar hairs are found also on the femora, coxæ and metapleuræ. The metatarsal joint of the hind legs is also kilated or enlarged and is furnished with rows of stiff hairs on its lower surface. In the male the hind tibiæ and metatarsi are slender and bear only short, sparse, straight hairs and this is true also of the coxæ and metapleuræ. The presence of Stylops in the abdomen of the female diminishes the development of the pollen-collecting apparatus to such a degree that the hind legs become like those of the male. The reverse occurs in stylopized males, the organs under consideration becoming more enlarged and approximating to the female type in their pilosity. The modifications in this sex, however, are rarer than in the female and in both sexes they vary greatly in different stylopized individuals. (3) The frontal furrow near the internal orbit of the eyes, which s filled with velvety pubescence, is well-developed in the normal female, but feeble or absent in the normal male. In stylopized Andrenæ this furrow may undergo diminution of development in the female and becomes accentuated in the male. (4) Although the female Andrena has 12-jointed, the male 13-jointed antennæ, there is no modification of the number of joint in parasitized individuals. The antennæ of the normal sexes may differ in the length of the second funicular joint. In one species, A. Trimmeriana, the second funicular of the normal female is as long as the two succeeding joints taken together, whereas in the normal male this joint is at most half as long as the succeeding

joint. In the stylopized male of this species Pérez found the second funicular attaining to two-thirds the length of the third joint and to this extent approximating to the conditions in the female. (5) The normal female Andrena bears a fringe of long hairs, the anal fimbria, on the edge of the fifth adbominal sternite, but this fringe is lacking in the normal male. Stylopization tends to suppress the development of the fimbria or causes it to disappear completely in the female and more rarely has the reverse effect on the male. (6) The sting, which is peculiar to the female, is reduced in size in the parasitized individuals, the copulatory organ of the male is also reduced in length and becomes narrower and less curved, while the paramera tend to become atrophied.

Pérez concludes from these observations that, so far as the secondary sexual characters of Andrena are concerned, the modifications induced by the Stylops are not merely attenuations, but actual inversions of development. "The stylopized Andrena, male or female, is not merely a diminished male or female; it is a female which takes on male attributes; a male that takes on the characters of the female."

The intimate correlation which exists between the structure and instincts of all organisms, leads one to look for instinct peculiarities corresponding with the morphological inversions described above. Pérez found only one stylopized female Andrena which had its hind legs charged with pollen, and he therefore concludes that the stylopized bees rarely or never forage or build nests like the normal females. Normal and parasitized bees of both sexes, however, visit flowers as this is not a unisexual instinct, and hence the triungulins produced by the Stylops have an opportunity to move off onto the plants, climb onto normal foraging bees and thus get transferred to the brood in incipient nests. In this way the perpetuation of the parasites is insured through a line of bees capable of nourishing them.

The internal changes due to stylopization have been studied by Newport (48), Pérez and Perkins (92). All of these authors find that the testes and ovaries are not destroyed by the parasite but are more or less reduced in size, in the male sometimes only on the side of the body bearing the Stylops. In the female the oöcytes

or ova degenerate in their follicles and are evidently quite incapable of development, in the male there may be ripe spermatozoa in at least one of the testes. Perkins found motile spermatozoa in all the stylopized males which he dissected, and Pérez mentions a male of Andrena decipiens taken *in copula*, so that this sex may retain, at least occasionally, not only the normal mating instincts, but the ability to fecundate normal females. The parasites before maturity live on the fat-body and blood-tissue of their hosts and do not attack the other organs directly. These undergo partial atrophy through lack of nutrition. Observations similar to those of Pérez have been published by Saunders ('82) and Schmiedeknecht ('83).[3]

Turning now to Polistes, we find that in this genus the secondary sexual characters are in certain respects quite as clearly developed as in the andrenine bees, but as wasps do not collect pollen, the hind legs show no special modifications in the female. The following are the main external sexual differences observable in Polistes metricus: The male has a slender thorax and long, narrow abdomen. The antennæ are 13-jointed, with a long, slender funiculus, not enlarging towards its tip; the second funicular joint is little if any longer than the two succeeding joints taken together. The face is long and narrow, with a pair of longitudinal grooves running from the antennal insertions to the clypeus and separated by a prominent longitudinal welt or elevation. The clypeus is flat or even slightly concave and its surface is impunctate. The whole face and clypeus, the anterior surface of the antennæ to within a few joints of the tip of the funiculus, the anterior surface of the coxæ, femora and tibiæ, a series of transverse bands or spots on the abdominal sternites behind as well as including the first segment, are sulphur yellow. The two large ferruginous spots on the first abdominal segment are usually well-developed.

In the female the thorax is proportionally stouter and the

[3] Though the publications of these authors antedate the article above reviewed, we are not to infer that this implies priority of discovery. Pérez says that he originally called the attention of these investigators to the facts and had himself published a preliminary account of his researches as early as 1880 in the Revue Internationale des Sciences. Tome I.

abdomen is decidedly shorter. The antennæ are 12-jointed, with a shorter funiculus slightly enlarging towards its tip; the second funicular joint is nearly as long as the three succeeding joints taken together. The face is decidedly shorter than that of the male, the grooves and welt much less pronounced and the clypeus is convex and coarsely punctate. The face is black, with the internal orbit and sometimes portions of the clypeus, the anterior surface of the scape and of the two first funicular joints, the anterior surfaces of the tibiæ and apical portions of the femora, ferruginous. The sulphur yellow is restricted to the tarsi and the posterior border of the first abdominal tergite, and the ferruginous spots on the first abdominal segment are obscure or wanting. The wings are often somewhat more deeply infuscated than in the male.

In stylopized Polistes metricus of either sex I fail to find any modifications of a morphological character which could be definitely attributed to the presence of the parasites. A few of the more heavily stylopized females were abnormally small, but with these exceptions, all the wasps were of normal stature. No modifications of the antennæ nor of the structure and proportions of the face could be detected. A study of the coloration, however, yielded more positive results, but even here, owing to the great range of color variation to which P. metricus like all our other species of the genus, is subject, the results are not capable of very precise formulation. In the coloration of the face stylopized males show no tendency to approach the female. In 14 out of 25 heavily stylopized females I find the clypeus of the usual black or dark brown color; in the remaining 11 it is more or less ferruginous or yellow. Some specimens have the free border of this sclerite sulphur yellow or its whole surface ferruginous, or only its posterior border or sides of this color. One specimen has the clypeus ferruginous with a small black spot in the center. It would be possible to regard these cases as approximations to the male type of coloration due to parasitism, were it not that perfectly normal, unstylopized females not infrequently exhibit the same erythrism of the clypeus. I have not seen a sufficient number of P. metricus from different localities to be able to determine

whether the percentage of this modification is so much greater among stylopized than among unstylopized individuals as to show that it must be attributable to the influence of the parasites. I am inclined to believe, however, that it is part of a more general erythrism which affects also the abdomen of many parasitized individuals. This region, to a varying degree in such specimens, but undoubtedly to a greater degree in those that are most heavily stylopized, takes on in both sexes alike a distinct ferruginous tinge which is usually most pronounced towards the posterior borders of the tergites and sternites. Sometimes it may be very strongly developed as in one rather small female taken August 29, and bearing three male Xenos. In this case the second gastric segment is entirely ferruginous, with the exception of a black anteromedian triangle, and the posterior half of each of the remaining segments and the whole clypeus, except its anterolateral corners, are rich ferruginous. I have failed to notice in the legs, wings and antennæ of either sex in stylopized specimens any color modifications that could not be regarded as falling within the wide limits of normal specific variability.

The color modification here described is not confined to stylopized specimens of P. metricus. It has also been observed by Brues ('03) in two of the Texan species, P. rubiginosus and annularis. "The stylopized Polistes," he says, "can be recognized even before the heads of the pupa cases begin to appear between the sclerites of the abdomen, by their paler color. They seem never to become as darkly colored as normal specimens. This lighter color of parasitized specimens seems to apply only to the originally dark species, in P. rubiginosus there seems to be but slightly if any lighter coloration. In the specimens of P. annularis from which I raised Xenos, all of them females, the faded appearance is especially noticeable upon the dorsum of the abdomen. The first abdominal which is normally piceous with a narrow apical yellow band is in this case almost entirely bright ferruginous, or is ferruginous with the border yellow. The remainder of the abdomen is normally piceous, but the posterior margins of the segments, especially the second and third tend to become more or less broadly dull ferruginous in stylopized specimens."

There is also a modification of behavior in stylopized Polistes. Several observers have noticed that such individuals are more sluggish, that they fly about less actively, and Brues ('03) has found that they are less inclined to use their sting, probably because the voluminous parasites interfere with the exsertion of this organ. A similar inability is observed in queen honey-bees with ripe ovaries and in worker honey-bees with their crops full of honey. The peculiarities of behavior in stylopized wasps are such as would be expected in parasitized organisms for these almost invariably exhibit a general reduction of vitality due to malnutrition.

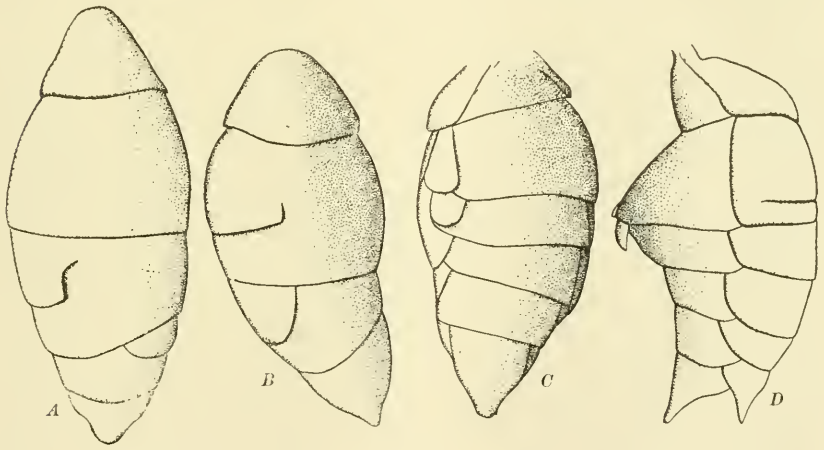


Fig. 2 Abnormal abdomens of Polistes metrica; *A* and *B*, dorsal; *C*, ventral; *D*, lateral view.

Among the unstylopized female Polistes taken at Colebrook there were three specimens with abnormal abdomens. Sketches of these are shown in Fig. 2. The segments in some cases were partially divided on either the right or left side, and in one case there were several supernumerary sclerites. It might be inferred that these abnormalities were the result of stylopization, for although no Xenos were found in the specimens, these parasites may have been present in the larvæ from which the anomalous individuals developed. I doubt this, however. At any rate, the anomaly in question is not peculiar to wasps that are subject to

stylopization or indeed to insects. Janet ('03) describes and figures a very similar abnormality in Vespa rufa, an insect that is not afflicted with Stylops or Xenos, and Cori and Morgan ('92) show that similar abnormalities are not uncommon in earthworms and cestodes. In the case of Polistes the abnormality must be produced either in the early embryonic stages while the metameres are forming or at the time of the formation of the abdominal sclerites in the pupa.

We may conclude, therefore, that Xenos produces no modifications of the secondary sexual characters of its Polistes host comparable to those produced by Stylops in the bees of the genus Andrena, but merely a tendency to a reddish coloration of the abdomen and face, a tendency which, so far as the abdomen is concerned, is manifested equally by both sexes.

This general lightening of color in stylopized Polistes and its reddish tinge remind one at once of the similar changes observed by Pérez in Andrenæ, although in the latter insects it seems to be confined to the pilosity. Pierce ('09, p. 32), cites the following observations, which show that a similar change of color was long ago observed by Saunders in stylopized bees of the genera Prosopis and Hylæus: "Prosopis gibba occasionally exhibits irregular rufous patches on the abdomens of affected individuals (Saunders, '50). Prosopis rubicola exhibits color changes regularly. The nymphs of those Hylæi which are likely to produce the pale-colored specimiens (H. versicolor), which prove, as anticipated, to be only a variety of the H. rubicola consequent upon parasitic absorption, may usually be identified within one or two days of their final metamorphosis by assuming a yellow tinge, and may be set apart as certain to produce male parasites. (Saunders '52.)" It is not easy to account for this modification. Brues is inclined to believe that "the reason that the reddish Polistes are not affected, is that red is a more primitive color than piceous and that the color simply becomes arrested at this stage and does not tend to become so before the red stage." The question of the development of variations of color in the species of Polistes is a very complicated one, as Miss Enteman ('04) has shown, and a number of possible explanations of the erythrism of stylopized individuals might be

suggested. The ontogenetic explanation suggested by Brues is one of these, implying that a red stage precedes the brown or black of the mature form of dark species like P. metricus. This is borne out by the development of the color pattern in such species. On this view stylopization inhibits color development in an ontogenetically and presumably therefore in what corresponds to a phylogenetically earlier stage. A second explanation is, however, suggested by Miss Enteman's studies. These tend to show that the dark-colored races or species of Polistes are due to cold and moisture, the lighter yellow and red forms to heat and aridity. This seems to be clearly indicated in the distribution of the species, *e.g.*, in such extreme forms as the yellow P. texanus and the black canadensis. It is possible, therefore, that the erythrism of stylopized P. metricus, which in normal coloration is closely related to P. canadensis, is due to withdrawal of water from the tissues by the developing parasites. This does not contradict the ontogenetic and phylogenetic explanations but supplements them, if we suppose that the primitive yellow or red color cannot pass on to the piceous or black stage unless the tissues contain a sufficient amount of water. Miss Enteman has shown that the piceous or black color is in the form of pigment granules in the chitinous cuticle of the wasp's integument, whereas the yellow is deposited in the hypodermis. Erythrism is probably due, therefore, to a diminution in the cuticular pigment which permits the yellow hypodermal pigment to shine through. As both kinds of pigment are the result of metabolism in the pupa, we can see how a disturbance of metabolism either through withdrawal of water by the parasites or through other causes might lead to the deposition of a smaller amount of the black pigment and hence to erythrism.

It is more difficult to account for the absence of all modifications of the secondary sexual characters in stylopized Polistes, when such modifications are so evident in Andrena. We may, perhaps, account for this difference on one of the following hypotheses:

1. As will be shown in the sequel, complete extirpation of the gonads in young larval insects, has produced in the few species on which it has been performed, no appreciable effects on the development of the secondary sexual characters. This indicates that

these characters may be so fixed and so nearly independent of the gonads, except, perhaps, in the very earliest larval or late embryonic stages, as to remain quite unaffected in their development after the gonads have been completely removed. The degree of this independence may be supposed to differ in different insects and even in different individuals of the same species. It may be slight or almost absent in Andrena and very well marked in Polistes and this may account for the differences between the stylopized specimens in the two genera.

2. The difference in the manifestation of changes in the secondary sexual characters may, however, be due to ethological differences between the two genera. Andrena has only male and female forms and both under normal conditions are adequately fed in their larval stages. In Polistes the larvæ of the earlier broods in the annual series, as Marchal has shown ('96, '97) are poorly fed and as a result become sterile females, or workers. As imagines they maintain themselves in a sterile condition by appropriating very little of the food they collect to their own use, since they at once lavish it in feeding the succeeding broods. Hence the females of these earlier broods become sterile, in the first place through alimentary castration of the larvæ from which they develop, and in the second place, maintain themselves in this condition as adults through the nursing or nutricial function (nutricial castration). These peculiar phenomena will be more fully discussed in the second part of this paper. Owing to these two forms of physiological castration inhibition of the development of the reproductive organs is a common and normal occurrence in Polistes females, and the parasitic castration induced by Xenos would not be expected to produce somatic changes of such magnitude or of such a nature as Pérez has observed in Andrena, all the females of which are normally fertile mothers. In other words, the effects of the Xenos on their hosts is of the same nature as the alimentary castration to which all the earlier broods during the seasonal development of the Polistes colony are normally subjected, and this probably accounts for the absence of any specific effects on stature and structure and the evident ease with which the voluminous parasites are borne and tolerated.

In the case of the male Polistes the matter is not so readily explained, since this sex is not subjected to the two forms of normal physiological castration just mentioned. But it should be noted that the effects of stylopization on the secondary sexual characters of the male even in Andrena are rarer than in the female (*vide*, p. 384), owing to the fact that castration is much less complete in this sex, as both Pérez and Perkins have shown. This is, no doubt, also the case in Polistes, for the development of the testes requires much less food than does that of the ovaries, and the presence of the Xenos probably, therefore, has much less effect on this sex.

It has long been known that male puparia and adult female Xenos are found only in the late summer or fall brood of Polistes in the brood, namely, which consists of fertile females and males that are to mate and provide, after hibernation of individuals of the former sex, for the formation of new colonies during the ensuing spring. Brues ('05) captured on May 22 a large over-wintered female of P. rubiginosus containing a female Xenos nigrescens that gave birth to a lot of triungulin larvæ. Evidently, therefore, the larvæ of the wasp must be infested with triungulins in the spring, soon after the colony is founded. How come it then, we are led to ask, that the adult Xenos appear only in wasps belonging to the last or autumn broods? If these wasps really belong to so late a brood they could not become infested unless we suppose that the triungulins hang about the wasps' nest for a long period before entering the larvæ. As this assumption is very improbable, we seem to be forced to the conclusion that the wasps that bear the Xenos in the late summer really belong to early broods which have been greatly retarded in their larval and pupal development. Dodd ('06) and Howard ('08) have published some interesting observations which show that the larvæ of other insects (Lepidoptera, Formicidæ) parasitized by chalcidids are greatly retarded in their growth and development. If this occurs also in Polistes larvæ infested with Xenos, as seems probable, we may be able to account for the facts and understand how the single generation of Xenos manages to survive till the following spring to insure the perpetuation of the race in healthy,

incipient colonies of the wasps. The triungulins are, in all probability, carried to these colonies by healthy wasps from the flowers onto which they crawl from their mothers after hibernating in their hosts.

Since the foregoing paragraphs were written Pierce's fine monograph of the Strepsiptera has appeared ('09). This work contains such a full summary of all that has been published on this remarkable group of insects, together with so much new matter, that I should have thought it unnecessary to publish the preceding pages, but for the fact that they were written for the purpose of elucidating a problem which Pierce treats only incidentally. Of the many interesting facts contained in his paper I shall cite only a few which have an immediate bearing on the matters considered above.

The fullest statistics given by Pierce relate to two large colonies of Polistes annularis infested with Xenos pallidus. These colonies, which were collected at Rosser, Texas, September 23, together contained 1553 wasps, 1311 males and 242 females. Of these 266, or 17.1 per cent were stylopized, 259 being males and only 7 females. The highest number of Xenos observed in a single wasp was 15, and this occurred in a male specimen! Pierce also cites some statistics published by Austin (1882) on 50 Polistes metricus collected at Readville, near Boston, Mass., August 20, 1879. Of these wasps, 14 of which were males and 36 females, 9 or 18 per cent were stylopized (2 males and 7 females).

Pierce figures the abdomen of a male wasp (Leionotus (Odynerus) annulatus Say) which has the sclerites much distorted as in the P. metricus shown in Fig. 2. Concerning his specimen, which contained a female Leionotoxenus hookeri Pierce, he says: "It seems that in pushing itself out between the segments the parasite completely split the dorsal tergites of segments three, four and five and split segment two half way to the base. The parasite was located behind segment three." He cites the observations of Pérez on the effects of stylopization in Andrena and adds the following modifications observed by Crawford in specimens of Andrena crawfordi infested with Stylops crawfordi:

"1. Puncturation of abdomen less strong, punctures finer and sparser; especially noted on second segment.

"2. In females with male parasites the basal joint of the hind tarsi is narrower, approaching the shape of the corresponding joint of the male tarsi; this joint not noticeably narrowed in female with female parasites.

"3. Scopa of parasitized female thinner, plumosity shorter, not so silky.

"4. Out of six males with male parasites two show the second transverse cubital gone in both wings; one has stubs at each end, however, in right wing; one has the transverse cubital slightly interrupted in both wings. Out of about 110 nonparasitized males none show any variation.

"5. Out of 38 females with male parasites one has the left wing with three submarginals, the right wing with two submarginals; one has two submarginals in both wings but right wing with a stub of the nervure; one has first transverse cubital of the left wing one-half gone; forty-five nonparasitized females show no variation.

"None of the other salient alterations found by Pérez could be expected in this species because of the close resemblance of the two sexes. Andrena crawfordi is a very generalized bee."

Pierce also calls attention to a single parasitized specimen of A. advarians in his collection, with a spurious nervure in the third discoidal cell, and believes that parasitism may affect the tracheation of the wings, a modification not observed by Pérez.

II GENERAL CONSIDERATIONS.

By employing the word "castration" in a broad sense to mean any process that interferes with or inhibits the production of ripe ova or ripe spermatozoa in the gonads of an organism, and not merely in the concise original meaning as the sudden and complete extirpation of the gonads, we are enabled to bring together a number of interesting but hitherto rather scattered facts which have a bearing on the correlation of the primary and secondary sexual characters. An adequate consideration of these facts would go a long way, I believe, towards preparing us for a profitable study of the recondite problem of sex determination.

Owing to the limits of this paper and to the fact that the dependence of the secondary on the primary sexual characters in vertebrates has been recently analyzed by several authors, notably by Herbst ('01) and Cunningham ('08), I shall confine my remarks very largely to the arthropods. Taking the word "castration" in the broad sense suggested above, we may distinguish:

1. *Surgical, or true castration, i. e.*, the sudden and complete ablation of the male or female gonads, so that the organism is deprived of its primary sexual characters, if we do not include in this term also the gonad-ducts and copulatory organs. This operation is of the greatest experimental significance, since, when performed at the proper ontogenetic stage, it has been shown to lead in many animals to interesting modifications of the secondary characters of each sex.

2. *Physiological castration.* Under this head may be included at least three different forms of inhibition in the development of the gonads, leading to a failure of the individual to develop its primary sexual characters, or, in other words, to an inability to function as a male or a female. This inhibition is brought about by an insufficient supply of nutriment and appears as the result of a well-known law, according to which the organism provides in the first instance for the growth and differentiation of its soma and develops its gonads on the nourishment in excess of that required for normal growth in stature and the complete differentiation of the various tissues. The following three forms of physiological castration may be distinguished:

A. Alimentary castration. This term was originally given by Emery ('96) to the suppression of gonadic development through insufficient feeding of the organism during its larval life.

B. Nutricial castration. This term was first used by Marchal ('97) to designate the maintainance of the gonads in an undeveloped condition in the adult, owing to the latter's devoting itself to nursing the brood of other fertile individuals instead of itself taking on the reproductive function.

C. Phasic castration. I use this term, for lack of a better, to include all the cases in which the gonads are inhibited in their development by seasonal or ontogenetic (growth) conditions. This

form of castration is not sharply marked off from the two preceding but may be made to include them, since both alimentary and nutricial castration can be suspended during the life time of the individual and normal reproduction supervene.

3. *Parasitic castration.* This term was first introduced by Giard ('87, etc.) in a series of studies on crustacea. It refers to the suppression or destruction of the gonads by parasites. By enlarging the scope of Giard's definition we can distinguish two forms of parasitic castration:

A. Individual parasitic castration, which is induced in certain organisms when they contain parasites, and

B. Social parasitic castration, which occurs in ants when one colony in becoming parasitic on a colony of a different species eliminates the sexual individuals of its host.

A number of illustrations will bring out the fundamental resemblances between these different methods of suppressing the reproductive function and the resulting modifications of the somatic characters of the individual or of their equivalents in animal societies.

1. *Surgical castration.*

The pronounced modifications of the secondary sexual characters observed in vertebrates, especially in birds and mammals, from which the gonads have been removed during early life, or in which these organs have become diseased, have led some investigators to look for corresponding modifications in the secondary sexual characters of insects subjected to a similar operation.

One observer, Hegner ('08), has succeeded in castrating the embryos of a chrysomelid beetle (Calligrapha multipunctata) by removing the very young sex-cells as soon as they are segregated in the protoplasmic accumulation at the posterior pole of the egg during the formation of the blastoderm. Although Hegner's experiment, which consisted in pricking the chorion at the posterior pole and allowing the sex-cells to flow out, was successful to the extent of demonstrating that the embryo may continue its development after the operation, nothing but a few young larvæ

were obtained. The experiment therefore, throws no light on the question with which we are here concerned.

Much more important are the results of experiments performed by Oudemans, Kellogg, Meisenheimer and Regen in castrating larvæ.

Oudemans ('99) was the first to attempt surgical castration in insects. He removed one or both gonads from male and female caterpillars of the gypsy moth (Ocneria dispar) before the last and second last moults. About one-third of the caterpillars (30 out of 86) survived the operation and produced moths. From a study of these, the Dutch investigator concluded that castration has no influence, either on the external appearance, *i.e.*, on the secondary sexual characters, or on the behavior of the moths, since the castrated males copulated, though they had no spermatozoa, and the females, though they had no eggs, nevertheless stripped from their abdomens the mass of long hairs in which they normally oviposit. Females castrated only on one side laid eggs, and three normal females that copulated with castrated males, laid eggs which developed parthenogenetically.

Kellogg ('04) succeeded in castrating silk-worm caterpillars (Bombyx mori) after the second, third and fourth moults by burning out the gonads with a hot needle. This method was very inferior to that employed by Oudemans. Not only was the mortality of the caterpillars greater, to judge from Kellogg's remarks, but the complete destruction of the gonads was obviously much less certain. Like Oudemans, he failed to detect any modifications of the secondary characters of either sex in cases in which dissection of the adult moths proved that the gonads had been completely destroyed.

More recently Meisenheimer ('07) has carried out much more elaborate experiments than either of his predecessors, on about 600 Ocneria dispar caterpillars, of which 186 yielded imagines. The smallest caterpillars castrated were between the second and third moults, and about $\frac{3}{4}$ cm. long, but he also used those between the third and fourth and between the fourth and fifth moults. He was able to remove the gonads even before the second moult but the larvæ were too delicate to survive the operation.

Three series of operations were performed: first, the removal of both gonads; second, the removal of the gonads together with the gonad-ducts; and third, the transplantation of testes into female and of ovaries into male caterpillars. The transplantation of ovaries was more easily performed than that of the testes. In these cases the transplanted organs not only developed to their normal size, but the ovaries in some cases even united with the male vasa deferentia. In one case a single transplanted ovary united with one of the vasa deferentia, and as the testes of the opposite side developed, an artificial hermaphrodite was produced. Meisenheimer describes the results of his operations as follows: "Oudemans' and Kellogg's experiments established the fact that the removal of the gonads exerts no influence on the secondary sexual characters. My results agree with these to the extent that in my experiments the originally male caterpillar always produced a male moth, the female caterpillar a female moth. The general habitus of the respective sex was always perfectly preserved, both in the form of the body, the structure of the antennæ and the coloration of the wings, and this was true of the operations, both in the case of the castrated moths and of the artificial hermaphrodites. But on examining, in a comparative way, the material obtained, a certain effect of the operations seems, nevertheless, to be noticeable. The moths subjected to the two kinds of operation may be arranged in series, which in the males vary from dark to light forms and pass over in the females from a whitish to a darker color." But, as Meisenheimer observed, there is considerable color variation in both sexes of normal gypsy moths, and this was true also of his control series, though he believed the variations to be greater in those developed from operated caterpillars. The specimens with transplanted organs, however, showed no greater modification than those of the castrated series. It is especially noteworthy that in the cases of transplantation there was no change in the copulatory or other organs, though these had not yet developed at the time of operating. Hence, although Meisenheimer made artificial hermaphrodites, he did not succeed in producing artificial gynandromorphs.[4]

[4] Unfortunately I was unable to secure a copy of the first part of Meisenheimer's final monograph ('09) till after the manuscript of my paper had gone to press. The review here given of his experiments is, therefore, inadequate.

It will be noticed that the preceding experiments were performed only on holometabolic insects of the order Lepidoptera. As such experiments on ametabolic insects might be expected to yield different results, it is interesting to record that Regen ('09, '10) has recently succeeded in castrating crickets (Gryllus campestris L). In his first paper he gives us little more than an orientation experiment performed for the sake of determining whether the insects would survive the operation, but his second contribution brings ampler and more satisfactory data. In order to perform the operation he narcotized the crickets with CO_2. The testes were removed from 40 males (20 in the second last and 20 in the last larval instar), and the ovaries were removed from 10 females in the last instar. These 50 individuals were released in the open field and each returned to the burrow which it is in the habit of occupying throughout its larval life. The operated individuals were marked by cutting off portions of their wings, and near their burrows stakes were placed with records of the necessary data. After the crickets had reached maturity Regen recovered 9 males that had been castrated in the second last, 13 of those castrated in the last larval instar, and 6 females. The insects were left in their burrows. Ten days later he found that the crickets had changed burrows and there was a tendency for them to associate in pairs, each consisting of a male and female occupying a hole in common. Several individuals had migrated to other parts of the meadow in which Regen experimented, but he succeeded in capturing and placing in a terrarium 10 males (4 castrated in the second last and 6 in the last larval instar) and one female On these specimens he made the following observations:

"1. Nine imaginal males, part of which had been castrated during the last and part during the second last larval instar, chirped throughout the remainder of their lives in as lively and shrill a manner as normal males. Only one of the males, which had been castrated in the last larval instar, chirped feebly and at rare intervals.

"2. The behavior of the castrated males towards the females was the same as that of normal individuals. They enticed the females with their shrill stridulation and when a female approached,

emitted a soft, whirring sound, and tried to affix their spermatophores to her, for

"3. The glands which secrete the spermatophore envelopes produced these structures up to within a short time of the death of the crickets and therefore performed their function independently of the testes.

"4. In external appearance the spermatophore envelopes of castrated males were in all respects like those of normal males (in some cases they were somewhat smaller), and contained a white secretion, which was less abundant than in normal spermatophores.

"5. The markings of the anterior wings, or tegmina and the development of the stridulatory organ showed no modifications.

"6. The females were unable to distinguish between normal and castrated males. They followed the call of the latter, mounted their backs and permitted them, as if they were normal males, to affix their spermatophore envelopes near the genital orifice.

"7. The castrated female behaved like one that had not been castrated. She thrust her ovipositor into the earth and made motions like a normal female, so that she had every appearance of desiring to oviposit. As time went on this "oviposition" became abnormal, as the female kept on thrusting her ovipositor into the earth but only to a slight depth."

Regen assured himself of the completeness of castration in these crickets by dissection and by examination of the spermatophores, which were found to contain no spermatozoa. He also kept a series of castrated individuals in captivity from the time of operation, and when these reached maturity they were found to behave exactly like the individuals that had been permitted to mature in the field. His experiments, therefore, gave results in complete harmony with those of Oudemans, Kellogg and Meisenheimer. It must be admitted that his insects were all castrated in rather late stages. He informs us, however, that during the summer of 1909 he successfully castrated a number of much younger larvæ, measuring only 5 to 8 mm., and that these had grown to a length of 20 mm., by December 1909 when he wrote his second paper. At that time the females were readily distinguishable

from the males by their ovipositors. He intends to remove the spermatophore glands from some of the males of this series and also from some uncastrated males and to report on the results in a further publication.

2. *Alimentary Castration*

The best examples of this form of castration are to be found among the social Hymenoptera, *i.e.*, among the social wasps, bees and ants. In these insects the majority of the female larvæ in each colony become what are called workers, because they are fed on a limited diet, grow very slowly, pupate more or less prematurely and hence as adults, or imagines are smaller in stature than the normal females of their respective species. These workers are also distinguished by other morphological and ethological peculiarities. Owing to their inadequate nourishment as larvæ, their ovaries are, as a rule, in a very rudimentary condition. Very striking examples of this alimentary castration are seen in the incipient colonies of ants, while the mother queen is bringing up her first diminutive brood of workers, in the species of Carebara, the queens of which are more than 1000 times as large as their sterile offspring, and in Pheidologeton, in which there is nearly as great a difference beween the stature of the queen and that of the smallest workers. In bumblebees, honey-bees, social wasps and most ants this difference is less pronounced, but it is nevertheless perceptible and clearly traceable to larval starvation. Opinions differ as to whether the other characters peculiar to the worker forms of these insects are the result of underfeeding, but it is evident that none of these can be regarded as an approach to the male type of structure. In other words, notwithstanding the very decided inhibitory effect of larval starvation on the development of the ovaries in the adult workers of the social Hymenoptera, the soma does not tend to become like that of the male, but merely departs to a greater or less degree from that of the female type. This departure is usually in the direction of greater simplification and is most pronounced in the ants, the workers of which are wingless, have a smaller and much simpler thorax and smaller eyes and ocelli.

The social Hymenoptera, however, are not the only insects which practice alimentary castration. A very interesting case is also seen in certain aphids of the genus Phylloxera, *e.g.*, in the Ph. caryæ-fallax recently studied by Morgan ('09). The stem-mother, or fundatrix of this insect makes and inhabits a hollow gall on hickory leaves. She lays numerous eggs which may give rise to two kinds of offspring. The eggs first deposited produce individuals that grow up to form the wingless sexuparæ, (Fig. 3*A*),

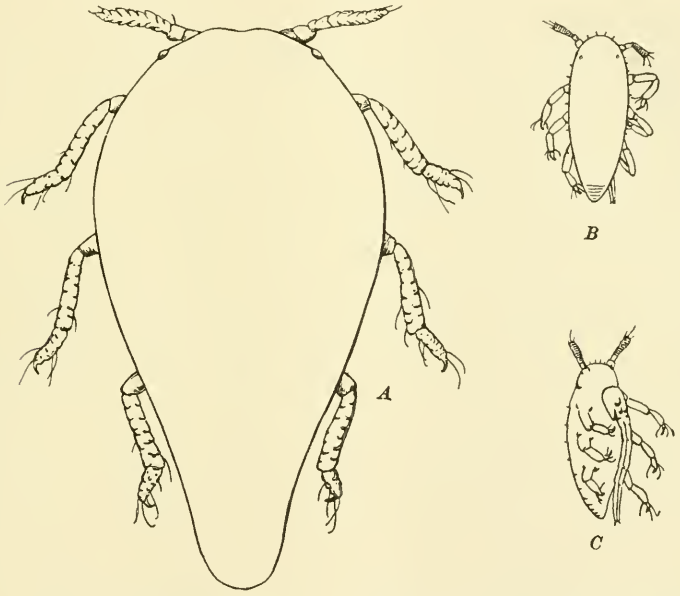


Fig. 3. Large wingless female of Phylloxera caryæ-fallax; B and C, dwarf females of same, drawn to same scale as A. (After T. H. Morgan.)

while the eggs laid later give rise abruptly to very small females, (Figs. 3*B* and *C*), which Morgan calls "supernumerary or dwarf females." These he describes as follows: "In the larger galls as many as 46 eggs may produce the large individuals, and then the smaller series abruptly begins; while in the smallest galls only one to three or four or more large individuals are produced when the small series begins. There seems to be here not a predetermined number of large and dwarf females, but the conditions

of life determine when the one kind ceases to be produced and the other begins. The two types of individuals must, however, be predetermined by alternative possibilities possessed by each egg. The supernumerary or dwarf females differ from their large wingless sister-forms, and from the young of the latter in a number of points. The shape of the body is entirely different and resembles that of the sexual male; but it differs from the male in two important respects; first, the dwarf individuals have a very long proboscis which in this species is absent in the male; second, there are no testes within the abdomen as in the males, where they form a relatively enormous mass. Otherwise the dwarfs are so similar in external form to the sexual males that their true nature was uncertain until they were studied in serial sections. These showed the absence of the testes and the presence of rudimentary ovaries and ducts resembling those of immature parthenogenetic females. There was nothing to indicate that the dwarfs could become sexual females. In fact the latter contain each an enormous egg when they hatch." Morgan believes that the dwarf females "are destined to a brief existence, and die without progeny," and he gives good reasons for supposing that they owe their origin to inadequate feeding of their parents. In other words, we have here a case of alimentary castration differing from that of the social Hymenoptera only to the extent that the mother insect provides her egg with an inadequate amount of yolk instead of feeding the larva from day to day on an insufficient amount of food. The resemblance of the dwarf females of Phylloxera caryæ-fallax to the workers of ants and other social insects is very striking, although it seems not to have been noticed by Morgan.

Perhaps the well-known "high" and "low" types of male in many insects, notably of the Scarabæidæ and Lucanidæ are to be regarded as the results of larval feeding. If this is the case, the low males may present examples of alimentary castration. This peculiar male dimorphism certainly bears more than a superficial resemblance to the female dimorphism of the social Hymenoptera. These may, indeed, be said to have high and low females, which, like the corresponding forms of the opposite sex in Scarabæidæ,

are sometimes connected by intermediates In ants the soldiers and desmergates represent the intermediate forms.[5]

But no one, to my knowledge, has studied the testes of beetles with dimorphic males, with a view to ascertaining whether these organs are more imperfectly developed in the low than in the high individuals. The low males undoubtedly approach the female in form, and might, therefore, be said to assume the secondary characters of this sex, were it not for the consideration that in a large number of scarabæid and lucanid species and genera both sexes have the same simple form. This indicates that the low male simply fails to develop its secondary sexual characters and hence returns to the ancestral type of the species in which these characters were either very feebly manifested or were altogether absent. G. Smith ('05*a*) has shown that in the Scarabæidæ and Lucanidæ, as well as in certain crustacea (Tanaidæ), "the differentiation into high and low males within the limits of a species has widely influenced the progressive differentiation among the different closely related species of many groups." This is somewhat more clearly expressed by saying that there are also high and low species in certain groups, the larger species of certain genera having a more pronounced male dimorphism than the smaller closely allied species. This is also true of the sexual dimorphism of female ants, as is seen in such genera as Solenopsis and Camponotus and among the genera of the subfamilies Dolichoderinæ, Camponotinæ and Myrmicinæ. It will be shown in the sequel that there is also another way of accounting for the "high" and "low" forms of some insects.

In this connection, I may briefly consider two cases which, if correctly reported, would appear to represent a complete loss of the reproductive organs by alimentary castration carried back into the early larval or embryonic period. Adlerz ('86) and Miss Bickford ('95) failed to find any traces of ovarian tubules in workers of the common pavement ant, Tetramorium cespitum. If this negative observation be correct, the workers of this ant must be regarded as utterly sexless. In my opinion, however, renewed investigation

[5] For a fuller account of the conditions in these insects the reader is referred to my paper on polymorphism ('07).

is required to establish the truth of this statement. The other case is even more doubtful. Silvestri ('06) recently described Copidosoma truncatellum, a chalcidid which is polyembryonic and infests the eggs and caterpillars of moths belonging to the genus Plusia, as possessing two very different larval forms. One of these he designates as "asexual" and states that it lacks every trace of the reproductive organs. It is very unlike the ordinary sexual larva in having a large head, well-developed mandibles and a very slender nematode-like body, and never lives beyond the larval stage. Silvestri believes that it has been developed for the purpose of breaking down the tissues of the host caterpillar and of thus rendering them more easily assimilable by the sexual larvæ which alone develop into imagines. The following considerations seem to me to cast considerable doubt on this interpretation: First, the asexual larvæ figured and described by this investigator are suspiciously like certain very young ichneumonid larvæ, and as their development is not satisfactorily traced to the same cell-masses from which the sexual Copidosoma larvæ arise, it is not improbable that the two larval forms really belong to two very different parasites. In other words, Silvestri's Plusia caterpillars were probably infested with ichneumonid in addition to Copidosoma larvæ. Second, I have been unable to find any larvæ of the asexual type in a number of American Plusia gamma caterpillars which were heavily infested with Copidosoma truncatellum. Third, as in many species of Chalcididæ larvæ of Silvestri's sexual type are able by their own endeavors to break down and assimilate the tissues of their host, it seems improbable that a single species should have developed a peculiar sexless and moribund larva for this particular purpose.

3. Nutricial castration

The abortive or rudimental condition of the sexual organs seen in the cases of alimentary castration may be normally prolonged and maintained throughout the adult life of the workers among the social Hymenoptera, when these insects are compelled to live on the slender remnant of food that remains to them after

they have satiated their queens and the young broods which are continually hatching from her eggs. Marchal ('97) has called attention to this condition in the wasps, and it has long been known to obtain in ants and the social bees, though the causal connection between the protracted immaturity of the ovaries in adult workers and their primary function as nurses had not been sufficiently emphasized. The form of castration which is thus produced is, however, not necessarily permanent. If the trophic status of a colony becomes highly favorable, or if the queen dies, the ovaries of one or of a number of the workers may undergo active growth and produce eggs capable of normal development. In such cases the workers may be said to usurp or to supplement the function of the queen, but owing to the fact that the adult insect cannot modify its external characters, there is no visible difference between the sterile and fertile workers, except in the size of the abdomen, and even this may be so slight as to escape observation. The primary cause of nutricial castration is to be sought in the instincts of the individual itself, whereas alimentary castration would seem to be attributable to the instincts of the individual's living enviroment, *i.e.*, to its nurses. This distinction, however, is probably more apparent than real, since as I have suggested in a former paper ('07), it is possible that the worker larva is from the beginning an organism predisposed to assimilate only a portion of the nourishment with which it is provided by its nurses. The growth and development of the larva obviously does not depend on the amount of food administered to it but on the character and rate of operation of its assimilating mechanism. A larva may be very voracious, but its tissues may be constitutionally unable to appropriate more than a limited portion of the food which enters its alimentary tract. The administration of highly assimilable food, as in the case of the "royal jelly" which is fed to the larval queen bee, may be, as I have maintained ('07), primarily for the purpose of accelerating the development of her ovaries, and the secondary characters of this insect, which are mostly of an abortive character (smaller sting, shorter wings, smaller hind legs) may be the result of this development.

Nutricial castration is not confined to the social insects but occurs also in mammals during the periods of gestation and lactation and in birds during incubation, as the result of a very similar inability of the organism to expend in reproduction the energies demanded by the exigencies of the nursing function.

4. Phasic Castration.

The forms of sterility which I include under this term, though temporary, cannot be sharply distinguished from the cases of alimentary and nutricial castration, since both of these may be abolished during ontogenetic development and yield to a fertile phase, as, *e.g.*, when worker ants become gynæcoid and nymphal termites become supplemental males and females. We may, indeed, say that the great majority of animals exhibit alimentary castration during their embryonic, larval and juvenile stages, but that this is not universally true is shown by the many examples of neotenia and pædogenesis scattered through the animal kingdom. There are, however, several cases of temporary castration which, though intimately dependent on the trophic condition of the individual nevertheless do not properly fall in the categories previously considered. The following may serve as examples:

A. Many hermaphroditic animals are protandric, *i. e.*, develop only their male reproductive organs at a very early stage and do not mature their female reproductive organs till after the testes are partly or wholly exhausted. Some of the most extreme cases of this phenomenon are seen in the epicarid crustacea and in the singular parasitic worms of the genus Myzostoma. In the crustacean Danalia the individual becomes a functional male while it is still a minute and active larva. Later this form attaches itself to the abdomen of a crab, loses its limbs, and develops a long proboscis which penetrates the tissues of its host. The abundant nutriment thus acquired enables the parasite to grow rapidly. Its ovaries then begin to enlarge, while the remains of its testes degenerate and are devoured by phagocytes, and the creature becomes a female. A very similar condition occurs, as I showed several years ago ('96) in certain species of Myzo-

stoma (*e.g.*, in M. pulvinar von Graff). In these striking examples the animal is only potentially hermaphroditic, since functionally it exhibits seasonal gonochorism through phasic castration of the ovaries during its youth and of the testes during its adult stages.

B. Geoffrey Smith ('05 *a*, '09) has called attention to a very striking form of phasic castration in decapod crustacea: "During the breeding season the males of Inachus mauritanicus fall

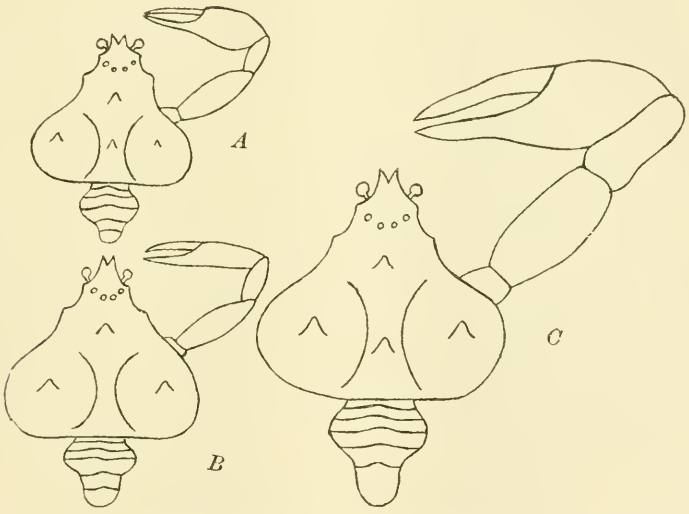


Fig. 4. Males of Inachus mauritanicus. *A* small breeding male with swollen chelæ; *B* non-breeding male, with slender chelæ; *C*, large breeding male with swollen chelæ. (After Geoffrey Smith.)

into three chief categories: Small males with swollen chelæ (Fig. 4*A*), middle sized males with flattened chelæ (*B*), and large males with enormously swollen chelæ (*C*). On dissecting specimens of the first and third categories it is found that the testes occupy a large part of the thoracic cavity and are full of spermatozoa, while in the middle-sized males with female-like chelæ the testes appear shrivelled and contain few spermatozoa. These non-breeding crabs are, in fact, undergoing a period of active growth and sexual suppression before attaining the final stage of devel-

opment exhibited by the large breeding crabs." This same condition was previously observed by Faxon ('85) in male crayfish belonging to the American genus Cambarus. Of course, the three stages distinguished by Smith are separated by moults. Obviously we have here a condition like that observed in many male fishes, amphibians and birds, which lose their secondary sexual characters during the seasons when they are not breeding. Smith regards the phenomenon as "obviously parallel to the 'high and low dimorphism,' so common in lamellicorn beetles," but this is a mistake, as Cunningham ('08) has shown, for we are here confronted with a case of seasonal sexual dimorphism. Nothing comparable to the condition described above is seen in insects, for the reason that these animals either do not mature their gonads till after they have attained their fixed and final imaginal instar, or if they become sexually mature as larvæ or pupæ (neotenic and pædogenetic aphids, cecidomyids, chironomids, etc.) they do not develop beyond this stage. It is not improbable, however, that insects which live several years in the adult stage and have seasons of sexual activity alternating with seasons of infertility, may exhibit great periodical changes in the size and development of the reproductive organs. I have been unable to find any observations on this subject in the entomological literature.

5. *Individual Parasitic Castration.*

The first zoölogist fully to appreciate the importance of parasites in suppressing the reproductive function and in incidentally affecting the somatic characters of their hosts was Giard. He published some twenty papers ('69–'02) on a great variety of cases which he observed not only among animals but also among plants. The cases to which he devoted most attention were the decapod crustacea, especially species of Stenorhynchus, Portunus, Carcinus, Cancer, Platyonychus, Eupagurus, Palæmon, Gebia and Hippolyte, which are infested with extraordinary cirriped and bopyrid parasites of the genera Sacculina, Portunion, Bopyrus, Probopyrus, etc. Within more recent years these studies have

been continued and deepened by Geoffrey Smith ('06, '09) on the spider crab Inachus mauritanicus infested with the cirriped Sacculina neglecta and by Potts ('06, '09) on hermit crabs (Eupagurus meticulosus) infested with the cirriped Peltogaster curvatus. A summary of the work of these two authors will not be out of place here, since they have reached rather definite con-

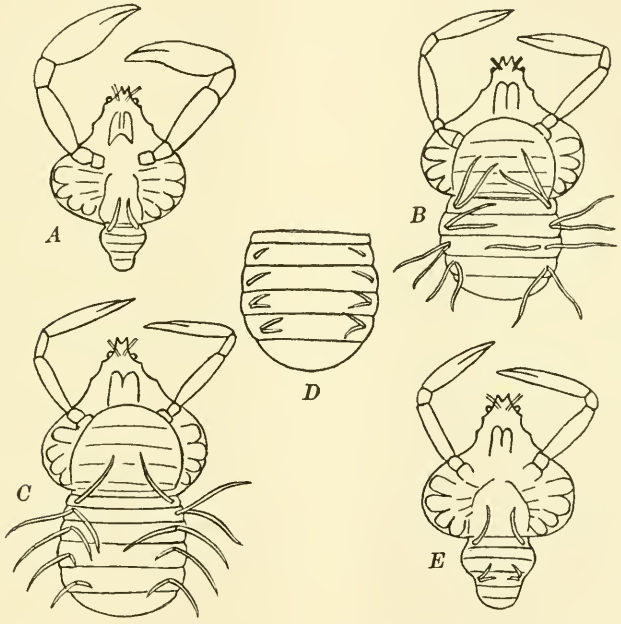


Fig. 5. Specimens of Inachus mauritanicus to show effects of parasitic Sacculina neglecta. *A* normal male; *B*, normal female; *C*, male infested with Sacculina (final stage); *D*, abdomen of infested female; *E*, infested male in an early stage of its modification. (After Geoffrey Smith.)

clusions not without a bearing on the various cases of parasitic castration in insects and other organisms to which I shall have occasion to refer.

According to Geoffrey Smith ('09) the abdomen of the normal male of Inachus mauritanicus "is small and bears a pair of copulatory styles, while the chelipedes are long and swollen (Fig. 5*A*). In the female (Fig. 5*B*) the abdomen is much larger and trough-

shaped, and carries four pairs of ovigerous appendages; the chelæ are small and narrow.

"Now it is found that in about 70 per cent of males infected with Sacculina the body takes on to varying degrees the female characters, the abdomen becoming broad as in the female, with a tendency to develop the ovigerous appendages, while the chelæ become reduced (Fig. 5*C*). This assumption of the female characteristics by the male under the influence of the parasite may be so perfect that the abdomen and chelæ become typically female in dimensions, while the abdomen develops not only the copulatory styles typical of the male, but also the four pairs of ovigerous appendages typical of the female. The parasitized females, on the other hand, though they may show a degenerate condition of the ovigerous appendages (Fig. 5*D*), never develop a single positively male characteristic. On dissecting crabs of these varying categories it is found that the generative organs are in varying conditions of degeneration and disintegration.

"The most remarkable fact in this history is the subsequent behavior of males which have assumed perfect female external characters, if the Sacculina drops off and the crabs recover from the disease. It is found that under these circumstances these males may regenerate from the remains of their gonad a perfect hermaphrodite gland, capable of producing mature ova and spermatozoa. The females appear quite incapable, on the other hand, of producing the male primary elements of sex on recovery any more than they can produce the secondary."

The following account is quoted from Pott's summary ('09) of his own studies on the modifications induced in Eupagurus by Peltogaster and of Smith's observations:

"The difference between the sexes of Eupagurus is shown only in a couple of external characters, the position of the generative apertures (as in all Decapods) and the character of the abdominal appendages. The abdomen of the hermit crab is furnished on one side only with a few appendages, insignificant, but with definite functions. It is in the female that we see the full development of the appendage as a swimmeret with two equal branches, the inner one provided with long hairs affording a secure anchorage for

countless eggs while the outer one is of equal size in both sexes, and in both by its paddle-movement maintains respiration currents in the shell. No use has been found for the outer branch in the male and so has become quite rudimentary, but the effect of the parasite Peltogaster is to stimulate the growth of this rudiment. There is of course great variability of response to this stimulus but those individuals which experience the maximum amount of change possess swimmerets exactly similar to those of a mature female, even in the assumption of the curious branched or barbed hairs which in this case can never bear eggs. As in the spider crabs so here, the female appeared incapable of the reverse change, and the large number of hermit crabs with typical female appendages and sealed genital apertures are undoubtedly to be regarded in part as modified males.

"A protest will conceivably be uttered against the attribution of a special sexual significance to the development of typical swimmerets in the male in both spider crabs and hermit crabs. It is of course well known that in the larval stages of these Crustacea biramous abdominal appendages are found in both sexes to be subsequently reduced or lost in the male. Lest this, then, be deemed a happy opportunity for applying the term "reversion" to this phenomenon I hasten once more to point out that when the male develops biramous abdominal swimmerets they are of the type associated with female maturity, and that the specialized nature of their nursing-hairs cannot well be associated with ancestral conditions.

"Both Sacculina and Peltogaster inflict sterility upon their host and apparently entire abortion of the gonad generally is the final consequence. On the external appearance of the parasite the eggs of the female shrink through absorption of their yolk and the formation of spermatozoa is after a time suspended in the male. The testis of the spider crab dwindles and disappears without undergoing any particular histological change; but in the hermit crab it is curious to note the presence of large cells with large nucleus and abundant protoplasm in sections of the testis. These instantly suggest ova in their appearance and call to mind the instances of the occurrence of such cytological elements as a nor-

mal experience in the testes of many animals. In sand-hoppers (Orchestia) to quote a well-known case (and there are many others in the Crustacea) spermatozoa are produced in the anterior part of the young testis while posteriorly the whole space is occupied by two or three large ova (*vide* Boulenger '08).

"The particular interest of the phenomenon in this case is its association with a definite cause, that is, parasitism. We are also able to come to some conclusion as to the degree in which such a condition can be called true hermaphroditism. Some striking evidence is offered by spider crabs which were once infected by Sacculina but which have outlived their parasite and recovered from its influence. Such crabs occur in nature in fair frequency and the only reminder of their former condition is the chitinous ring on the abdomen which surrounded the peduncle of the parasite. After the death of the external part of the Sacculina the root system may continue to exist in the host and it is only when this has disintegrated and been absorbed that regeneration of the gonads becomes rapid, for the still living roots repress the development of the sexual organs as effectually as the living parasite. A few crabs however were found in which the gonads had again attained full size and maturity. One was a female with a well-developed ovary and four were males only slightly modified externally, with glands producing large quantities of spermatozoa. The remaining four cases were remarkable for the crabs showed with a complete external hermaphroditism the corresponding gonads. In all four animals the reproductive gland consisted of a male part with ripe spermatozoa, and a female division with large pigmented ova. The ducts were usually absent, but one individual possesssed both vasa deferentia and oviducts. The sequel to these observations is given by the experimental evidence which Smith then obtained. It was attempted to destroy the parasite by removing the external part and the crabs so freed were kept under comfortable conditions for several months and the few survivors then killed. Regeneration had obviously occurred to a considerable extent, but the gonads were nearly always unisexual. In one individual alone, which was externally a hermaphrodite there was a gonad similar to those just described. In spite of the

comparatively small number of cases with fully formed hermaphrodite glands we are not going too far in definitely asserting a connection between their occurrence and parasitic influence, for bisexual gonads have to my knowledge never been met with in Decapod Crustacea under normal conditions.[6] But it thus appears that the curious condition in the hermit crab is an incipient stage corresponding to the perfect hermaphroditism of the "recovered" spider crabs, and if the action of the parasite in absorbing surplus nutrition were withdrawn the young ova in the testis of the hermit crab would become large and pigmented like those in the spider crab.

"These two cases have been described at some length as examples of *extreme modification*. In other Decapod Crustacea which are infected by the same parasite an effect is observable which is similar in kind but not in degree. The common shore crab of England (Carcinus) is commonly afflicted (if affliction it be) by Sacculina. Here again the male undergoes modification while the reverse change never occurs in the female. The narrow abdomen of the male is often exchanged at the moult after infection for one much broader but never attaining the full female width. One may look in vain, however, for any reduction of the copulatory styles or for the appearance of the smallest rudiments of swimmerets. The closure of the genital apertures nearly always follows parasitic attack in spider crab and hermit crab; but they never become blocked up in shore crabs with Sacculina. Yet the external change is apparently greater than that produced in the reproductive glands. Dissection in every parasitized male showed vasa deferentia of the characteristic milky white color due to countless masses of spermatophores all packed with spermatozoa. The testes though reduced, then, always remain in reproductive activity. The parasites which infect spider crab and shore crab are practically identical and presumably exert a very similar stimulus yet the results are markedly different. It is obviously the host which offers a different reaction in the two cases. In another

[6] In a footnote Potts states that "Calman in the recently appeared volume Crustacea of Ray Lankester's Treatise on Zoölogy refers to the unpublished observations of Wolleback on normal hermaphroditism in certain deep-water Decapoda."

crab (Eriphia) examined by Smith there was infection both by Sacculina and by a parasitic Isopod crustacean. Here the nature of the parasite governs the result, and crabs with Sacculina alone never showed the least trace of modification, while changes closely similar to those described above occurred in those which harboured the Isopod."

Geoffrey Smith ('05 *b*) has also described parasitic castration in Inachus dorsettensis by a sporozoön (Aggregata inachi) which lives in the intestine of the crab and induces modifications not unlike those induced by Sacculina. Smith says that of fifty males of I. dorsettensis examined, "seven specimens were clearly distinguished by having the flat chelæ characteristic of the females, while the abdomen was much broader than is the case in normal males of a corresponding size, thus converging on the female condition. In one specimen there was present on the under side of the abdomen a pair of swimmerets which are characteristic of the female, these appendages being altogether absent in the normal males." Dissection of these crabs showed the intestine "to be covered with cysts of Aggregata inachi, the body cavity was also full of liberated sporozoites, the hæmolymph having a milky appearance due to the crowded presence of these bodies. The testes were in all cases disintegrated, only the vesiculæ seminales remaining. Two modified males were also found to contain the cysts of Aggregata inachi, but in none of these males were there larger quantities of sporozoites in the hæmolymph, so that it appears that the hermaphrodite external characters are assumed by the infected male at the moult which follows the liberation of a large quantity of sporozoites." Smith made no observations on the infected female Inachus, as this sex is much rarer than the male.

The foregoing examples of parasitic castration in crustacea have been reviewed at some length, because they show the phenomenon in its most striking manifestation. Giard as early as 1888 (*b*) published a long list of other animals and plants known to be castrated by what he calls "gonotomic" parasites. The most interesting examples, apart from Andrena and the crustacea just considered, are the castration of the nemertean Lineus obscurus by the orthonectid Intoshia linea, of the planarian Leptoplana tremellaris

by Intoshia kefersteini, of the brittle-star Amphiura squamata by the orthonectid Rhopalura giardi and by a copepod (Fewkes '88), of the snails of the genera Paludina, Lymnæa and Planorbis by distome sporocysts (Distomum militare, retusum, etc.), of the crustacean Cyclops tenuirostris by larval distomes (Herrick '83), of the bumble bees (Bombus) by the extraordinary nematode Sphærularia bombi, and of the males of various North American squirrels and chipmunks (Tamias lysteri, Sciurus hudsonius and leucotis) by the bot-fly Cuterebra emasculator as described by Fitch ('59), Riley and Howard ('89) and Osborn ('96). Among plants Giard cites the castration of the fig by Blastophaga grossorum, of Melandryum album (Lychnis dioica) by Ustilago antherarum and various grasses by smuts, ergots, rusts, etc. The case of Melandryum and Ustilago which was repeatedly studied by Giard ('69, '87*a*, '88*d*, '89*a*) bears a curious resemblance to that of the male crab infested with Sacculina. The Melandryum is "normally diœcious. The young flower is hermaphrodite but in certain individuals the ovaries abort, in others the stamens remain rudimentary. When the parasitic fungus develops on a male plant, it fructifies in the stamens, but when it falls on a female plant, it seems at first as though it could not fructify and that the infested plant must profit accordingly. But this is not the case, for the plant develops its rudimentary stamens completely in order to permit the fructification of the parasite, just as the male Stenorhynchus enlarges its abdomen in order to protect the Sacculina fraissei."

Castration frequently occurs in plants through petalody, or petalomania, *i. e.* the conversion of stamens or carpels into petals, producing the well-known "double" flowers. Molliard ('01) has produced petalody experimentally in Scabiosa columbaria by artificially infecting the plant with the nematode Heterodera radicicola. And this investigator, Meehan ('00), Giard ('02) and Cramer ('07) cite a number of observations which indicate that petalody is often the result of infection of a plant with root-fungi. Veuillemin ('07) has observed in Lonicera infested with aphids a suppression of the carpels and a distinct androgeny of a certain number of the flowers.

Instead of stopping to review the various examples of parasitic castration cited by Giard in his paper of 1888, and in many of his later publications, it will be preferable to describe as briefly as possible a number of selected examples, especially some that have come to light more recently among insects. The stylopized Polistes and Andrenæ, having been adequately described in the first part of this paper, will be omitted.

Grassi and Sandias ('93) describe a remarkable case of parasitic castration in termites. They find that worker and soldier termites have the intestinal cæcum, which occupies much of the abdominal cavity, distended with enormous numbers of parasitic Protozoa belonging both to the Ciliata (Dinonympha, Pyrsonympha, Trichonympha) and to the Gregarinida. The Ciliata have been studied by several authors, notably by Leidy ('77, '81), Grassi ('85), Kent ('85), Porter ('97), and Dodd ('06). In termites infested with these parasites the reproductive organs, both male and female, remain small and undeveloped, apparently as the result of the pressure exerted on them by the distension of the cæcum. The parasites are absent in the very young termites and in the sexual forms, which are fed on saliva. Grassi and Sandias infer that the Protozoa must either be killed off or, at any rate, prevented from living and growing in the alimentary tract of saliva-fed individuals. These investigators are inclined, therefore, with some reservations, to regard the development of the two sterile castes in termites as the result of infection with protozoan parasites. This infection is, of course, readily brought about as the workers and soldiers are not fed on saliva like the sexual forms but on dead wood and on the fæces of individuals belonging to the same castes.

The researches of Grassi and Sandias have received a certain amount of confirmation from Brunelli ('05), who finds that queens of Calotermes flavicollis and Termes lucifugus sometimes become infested with the parasitic Protozoa, and that when this happens the young oöcytes in their ovaries degenerate. Calotermes queens are more susceptible to this form of castration then the queens of Termes. Brunelli explains the winged soldier observed by Grassi and Silvestri's ('03) 48 workers of Microcerotermes struncki with

well-developed reproductive organs (40 females and 8 males), as being instances of fertility brought about by a disappearance of the Protozoa through some unknown cause. Such fertile soldiers and workers would be comparable to the "recovered" spider crabs above described, except that there is no tendency towards hermaphroditism.

It is not altogether improbable that the high and low males among the Scarabæidæ, Lucanidæ and Forficulidæ are produced

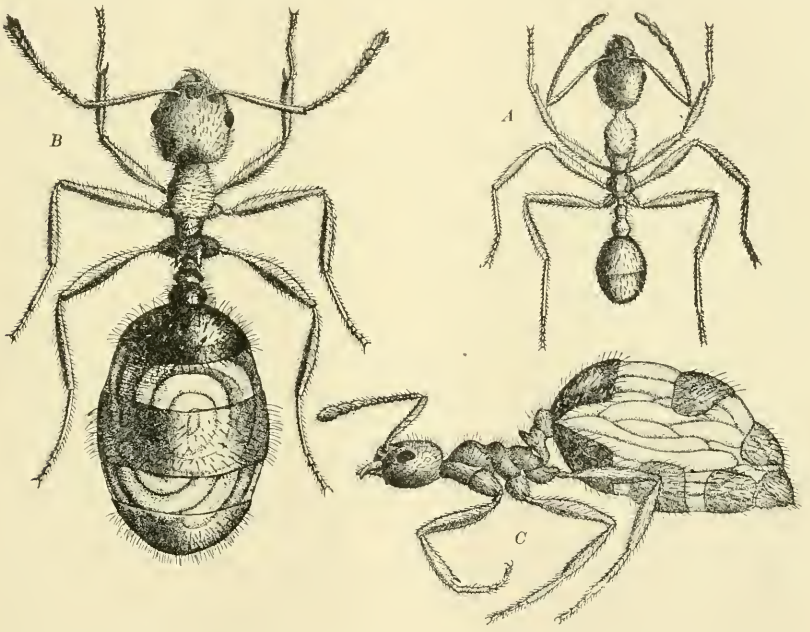


Fig 6. *A*, normal worker of Pheidole commutata; *B* and *C* mermithergate of same in dorsal and l teral view.

in some such manner as the workers and soldiers of termites. It is certainly suggestive that all three of these families of insects live on decomposing vegetable substances and in situations where they become very readily infected with gregarines. Giard ('94*a*) has given good reasons for supposing that the high and low males of Forficula, which were made the basis of a statistical study by Bateson (92), are produced by differences in the number of gregarines they harbor in their alimentary tract. The French

observer says: "It is, indeed, possible to predict from the length of its forceps whether or not a male Forficula possesses gregarines and whether these are present in greater or lesser numbers. Since these parasites produce a diminution of a secondary sexual character, that is, the length of the forceps, without bringing about absolute sterility (complete castration being exceptional), it not infrequently happens—and this is the case both on the beaches of Wimereux and on the Farne Islands—that the individuals with short forceps, namely, those containing parasites, are more numerous than the individuals with long forceps." Giard is inclined to believe that similar conditions may obtain in such beetles as Xylotrypes gideon, Oryctes nasicornis and other Scarabæidæ with high and low males. The low males of these beetles, however, are not to be regarded as having acquired female characters, but as having lost the male characters, so that, as Giard remarks, the "infested individuals are generally pædomorphic as compared with the normal form."

In two of my former papers ('01, '07) I described a peculiar case of parasitism in a Texan ant, Pheidole commutata. The larvæ of this insect are occasionally infected with nematodes of the genus Mermis and develop into peculiar forms, which I have called mermithergates (Figs. 6*B* and 6*C*). These are much larger than the normal workers (Fig. 6*A*), which they nevertheless resemble in the structure and small size of the head, although they possess small ocelli and in this respect resemble the queens. In thoracic structure they approach the soldier form while the gaster is enormously distended with Mermis and retains scarcely any vestiges of the fat-body, reproductive organs and other viscera. The behavior of these parasitized individuals is also peculiar, since they never excavate the soil, nor care for the brood like the normal workers, but run about in a state of chronic hunger, begging food from their uninfested nest-mates. Emery ('90, '04) has recorded the occurrence of mermithergates in quite a series of neotropical ants, including Pheidole absurda and several Ponerinæ of the genera Odontomachus, Neoponera, Ectatomma, Pachycondyla and Paraponera.

In the cases described by Emery and myself only the worker

forms were infested and modified by the Mermis, but Mrázek ('08) has recently shown that the virgin queens of the European Lasius alienus may become infested with this worm and that when this occurs the insects develop abnormally small wings (Fig. 7*B*). These individuals, or mermithogynes, as Mrázek calls them, have been seen by other investigators and described as brachypterous to distinguish them from the normal macropterous individuals of the species.

After seeing Mrázek's paper I examined a small collection of seven brachypterous and as many macropterous females of Lasius neoniger (a form closely related to alienus) which I had taken from a single colony near Manitou, Colorado, August 9, 1903. Three of the short winged individuals were dissected and each was found to contain a large coiled Mermis, 53 to 55 mm. long, which filled out the whole abdomen, so that in the living individuals there could have been little left of the reproductive organs and other viscera. There is nothing unusual in these females except the small size of their wings, which measure only 6 to 6.5 mm. in length, whereas those of normal L. neoniger females measure 10 to 11 mm. These observations show that the queens of our American Lasii may be affected by Mermis in exactly the same manner as the queens of the related European species.

The species of Mermis are not, however, the only known gonotomic nematodes. A much more extraordinary form is Sphærularis bombi, which has been known ever since the days of Réaumur (1742) to produce sterility in the hibernating queens of bumblebees. According to Leuckart ('87), who has written the best and apparently also the most recent account of Sphærularia, infested bees are sometimes found, "which have not a single mature egg in their ovaries. Structurally these organs are perfectly developed and have ova in the blind ends of their ovarioles, but ripe eggs are lacking. In other specimens one may find in addition to the young, also some ova of perfectly normal dimensions." He says that he has "never seen an infested queen which had the ovarioles as uniformly and richly provided with eggs as are the ovaries of healthy bumble-bees at the same season. As a rule, one finds only a few eggs, sometimes only a single egg." These

bees are therefore unable to found colonies, according to Schneider and Leuckart. They keep flying about till late in June and then die, whereas uninfested queens have started their colonies and no longer fly at large after the beginning or middle of May. Sphærularia occurs only in the queens, and has never been found in those that have become mothers of colonies. It would be interesting to know whether the colony-founding instincts of

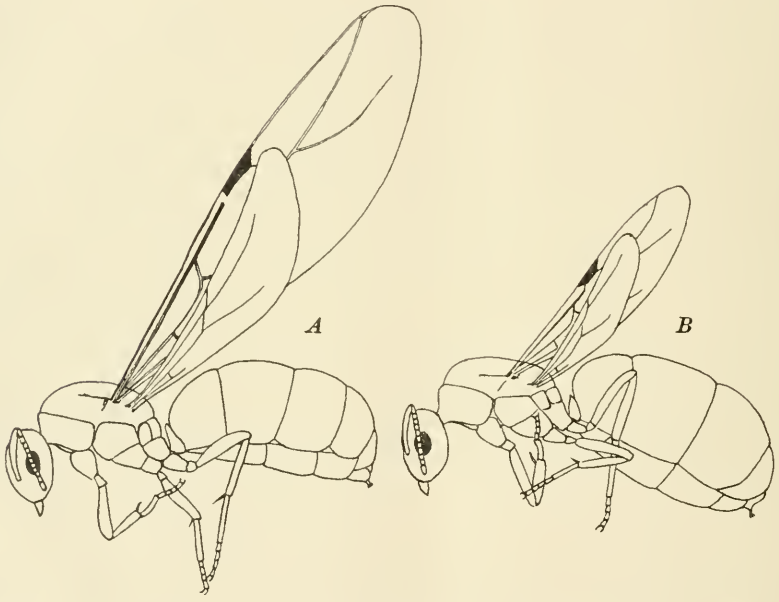


Fig. 7. *A*, normal female of Lasius alienus; *B*, mermithogyne of same species (After Mràzek.)

infested queens show the same tendency to atrophy as the ovaries. As the bees become infected in their imaginal instar, apparently while seeking their winter quarters, the parasites can produce no modifications in the external characters.

The Lasius mermithogynes described above recall some observations of Künckel d'Herculais ('94) on Algerian grass-hoppers (Stauronotus maroccanus and other species) infested with flies of the genus Sarcophaga. The maggots of the flies are entoparasitic, devouring the fat-body, and, according to Künckel d'Herculais, also absorbing the oxygen dissolved in the blood-plasma of

their hosts. The results are an atrophy of the reproductive organs (parasitic castration) and a weakening of the wing-muscles, so that the grasshoppers have a disinclination to fly. For this latter condition, which is described as a "kind of rhachitis," Künckel d'Herculais suggests the name "aptenia." Like the brachyptery of the Lasius mermithogynes, it points to an intimate correlation between the development of the reproductive organs and the wings, a correlation which is also clearly demonstrated in most insects by the coincident maturation of the former and full development of the latter organs at the beginning of the imaginal instar.

The extensive literature on entoparasitic Diptera and Hymenoptera, if carefully searched, would probably yield a number of accounts of parasitic castration. Pantel ('09), in an important paper, distinguishes both direct and indirect parasitic castration as the result of the infestation of lepidopteran larvæ with the larvæ of tachinid flies. In the former case the fly larvæ live in the testes of the lepidopteron and destroy the gonadic elements directly. In the latter the gonads suffer atrophy through the action of the parasites on the other viscera. The only cases I have found in which the host shows a modification of its external sexual characters as the result of such castration, are the homoptera Typhlocyba hippocastani and douglasi, which are described by Giard ('89*b*, '89*d*) as being infested with a dryinid hymenopteron, Aphelopus melaleucus and a pipunculid dipteron, Chalarus (Ateloneura) spuria. The females of both species of Typhlocyba, when castrated by Aphelopus, have the ovipositor much reduced; the Chalarus alone seems to have less effect on this organ. The penis of the male T. douglasi is little modified by either of the parasites, but in T. hippocastani infested with Chalarus, this organ shows a decided reduction in size and simplification of structure so that the specific characters become profoundly modified. None of these modifications, however, indicates any tendency to take on the characters of the opposite sex.

6. Social Parasitic Castration

This category is not sharply marked off from the preceding, for if we define it as including those cases among social insects

in which the individuals that represent the reproductive organs (*i.e.*, the males and queens) of the colony considered as an organism of a higher order, are castrated by parasites, we should perhaps include also the Lasius colonies containing mermithogynes and the queens of Bombus infested with Sphærularia described in the foregoing paragraphs. But in these cases it is merely prospective colonies, so to speak, which are castrated, since neither the mermithogynes nor the parasitized Bombus queens have as yet become mothers of colonies. For this reason I have treated them as cases of individual parasitic castration. Here belongs also the production of pseudogynes in Formica colonies infested with the peculiar myrmecophilous beetles of the staphylinid tribe Lomechusini (Lomechusa and Xenodusa) which I have considered at length in a former paper ('07). These beetles tend to suppress the development of the annual brood of virgin queens since the worker ants of parasitized colonies either neglect the queen larvæ or endeavor to convert them into workers, after the period during which this change can be successfully accomplished has passed. The results of this behavior is the production of the nonviable pseudogynes and the gradual degeneration of the colony. In this case also the colony is not castrated, but the mothers of prospective colonies may be said to suffer from misapplied alimentary castration.

Leaving all these cases out of account we have left only those in which a parasitic colony of insects prevents the development of or destroys the fertile sexual individuals of the host colony in which it lives. As parasites of this type I may mention the various slave-making ants (Formica sanguinea and Polyergus rufescens and their various varieties and subspecies), the temporary social parasites (Formica rufa, exsecta, exsectoides, etc.) and the permanent social parasites of the genera Anergates, Wheeleriella, Epipheidole, Sympheidole and Epœcus. There are other social parasites that do not destroy the reproductive individuals of the host colony, for example, the bees of the genus Psithyrus, which live in the nests of bumble-bees, and among ants such species as Leptothorax emersoni, Formicoxenus nitidulus and Harpagoxenus sublevis. Still other ants, such as the species of Strongylognathus,

do not destroy the queen of their host colony (Tetramorium cespitum), but since the workers of this colony prefer to rear the small sexual forms of the parasites instead of their own bulky males and females, the development of future colonies of the host species is rendered impossible and we have here again a case of prospective social castration.

The conclusion which we reach after marshaling this long series of illustrations of the various forms of castration is that among insects the only case in which destruction or inhibition of the reproductive function clearly results in any modifications of the secondary sexual characters comparable to the modifications observed in vertebrates under like conditions, is that of the stylopized andrenine bees as described by Pérez. In all the other cases extirpation of or injury to the gonads may indeed result in modifications of the somatic or secondary sexual characters, but the latter do not take on the peculiarities of the opposite sex. The most striking illustrations of the truth of this statement are the insects that have been surgically castrated. These show that the secondary sexual characters must be so independently and so immovably predetermined and at so early a period in the ontogeny that complete extirpation of the gonads during prepupal life fails to produce the slightest curtailment or modification either in the secondary sexual characters or in the sexual instincts of the adult insect. This conclusion renders it imperative to reinvestigate the cases of stylopization in the andrenine bees for the purpose of ascertaining whether Pérez's interpretation is the only one which they will yield, especially since it has been shown in the first part of this paper that the study of stylopization in Polistes leads to a very different view and one in complete harmony with the other cases of castration in insects.

It is interesting to note that castrated crustacea, to judge from the observations of Giard, Geoffrey Smith, and Potts, show modifications like those of castrated vertebrates and not like those of the insects. This is in all probability due to the fact that the development of the primary and secondary sexual characters is gradual and continuous in the Crustacea and vertebrates, whereas both these characters in insects are arrested in their develop-

ment and remain unaffected by the surrounding processes of growth and differentiation till the imaginal stage is attained. In holometabolic insects the secondary sexual characters are, of course, segregated in the imaginal discs, or histoblasts, and even in hemimetabolic and ametabolic insects there must be a similar isolation of the cell-materials which will produce the somatic sexual peculiarities of the adult.

The opinion here advocated, namely, that in insects the primary and secondary characters are very loosely correlated during ontogenetic development or in a very different manner from what they are in vertebrates or even in the crustacea, receives indirect support from two interesting classes of facts. One of these classes comprises the anomalies known as gynandromorphs,which, though always rare, are nevertheless much more frequently found among insects than among any other animals. These anomalies consist in combinations of male and female somatic characters in the same individual, usually in such a manner that the two lateral halves or the anterior and posterior portions of the body are of different sexes. In the former combination the reproductive organs may be hermaphroditic and correspond with the sex of the halves of the body in which they lie, but this is not always the case, and in anteroposterior, or frontal, or in mosaic,or decussating gynandromorphs, which exhibit an irregular mingling of the the sexual characters, the gonads may nevertheless be unisexual. Herbst ('01) and Driesch ('07) have emphasized the obvious inference that these various arrangements of the male and female characters cannot owe their origin to internal secretions, or hormones, and indeed all those who have speculated on the origin of these anomalies are unanimous in holding that they must arise either from peculiarities in the structure of the egg or from irregularities in its fertilitation or early cleavage stages at the very latest. Among recent speculations on the origin of gynandromorphism those of Boveri ('02) and Morgan ('05, '09) may be mentioned. Boveri believes that the gynandromorph arises from an egg which has segmented prematurely, so that the male pronucleus unites with one of the cleavage nuclei. Morgan is of the opinion "that the results may be due to two (or more) spermato-

zoa entering the same egg, one only fusing with the egg nucleus and the other not uniting, but developing without combining with any parts of the egg nucleus." These hypotheses have no very cogent facts to support them and I fail to see how they have any advantage over the hypothesis which was advanced by Dönhof as long ago as 1860, to the effect that the gynandromorph arises from the fusion of two eggs, only one of which, in the case of the honey bee, is fertilized. In its original form Dönhof's hypothesis is incomplete, but I believe that its plausibility is increased by addition of the following considerations. We may assume with Beard ('02), von Lenhossék ('03), Reuter ('07), Morgan ('09) and others that the gonochoristic Metazoa produce two kinds of eggs, male and female, which may or may not differ in size but differ in sex even as oöcytes. Now we know from zur Strassen's researches on Ascaris ('98) that two eggs may fuse and nevertheless give rise to a single embryo of perfectly normal structure though of twice the normal size. In Ascaris the fusion occurs after the oöcytes have reached their full growth, but a fusion of younger oöcytes would be, in all probability, not only more readily accomplished but lead to the formation of a single embryo of the normal size. The structure of the ovarioles of insects indicates that it would be a very easy matter for two young oöcytes to become enclosed in the same follicle, too easy, indeed, to accord, at first glance, with the fact that gynandromorphs are such rare anomalies. But if two female or two male oöcytes fused no gynandromorph would result, and the chances of either of these fusions of like oöcytes occurring would be quite as great as that of two oöcytes of opposite sex. If this be the way in which gynandromorphs arise, we should have to explain the occurrence of the lateral type of the anomaly by supposing that the plane of fusion of the two eggs becomes the median sagittal plane of the future insect, whereas in the frontal type this plane would be transverse to the longitudinal axis. Finally, in the mixed and decussating types we should have to suppose that the male and female egg-materials are mixed or interpenetrate one another to a variable degree. The hypothesis here sketched has the advantage of permitting of some slight cytological verification, for microscopic examination

of the ovarioles of a large number of Lepidoptera, which seem to present the anomaly in question more frequently than other insects, might reveal an occasional inclusion of two oöcytes in the same follicle or even various stages in their fusion. Or if hives are ever again found like the famous Eugster hive, in which so many gynandromorphous bees were produced, the cytologist will have an opportunity to test the hypothesis here advocated by a careful examination of the ovarioles of the queen.

But no matter what view we hold in regard to the origin of gynandromorphs, we are compelled to admit that they demonstrate the very early and rigid determination of the secondary sexual characters, the possibility of their complete development even when the gonads of the corresponding sex are lacking and their independence of internal secretions. To this extent they confirm the results obtained by Oudemans, Kellogg, Meisenheimer and Regen in their castration experiments. Indirectly they indicate that the insect egg not only has its primary sexual characters determined long before fertilization and independently of the later nuclear or chromosomal phenomena, but that even the secondary sexual characters are in some manner also predetermined at this early stage. Where great differences of stature are secondary sexual characters, as in phylloxerans, some aphids and rotifers, we find corresponding differences in the size of the male and female oöcytes. This is, of course, quite in harmony with the remarkable predetermination of the embryonic regions of the insect egg. Long ago Hallez ('86) and I ('89, '93) showed that in many insect eggs the regions corresponding to the ventral and dorsal, right and left, and cephalic and caudal portions of the embryo are clearly established long before the maturation divisions.

The second class of cases, which indicate that the primary and secondary sexual characters of insects may develop independently of one another, are found among certain species of ants, the males of which, though developing gonads and external genitalia of the usual type, have nevertheless become decidedly feminine in their secondary sexual characters. That this condition is an expression of degeneration seems to be indicated by the fact

that it occurs only in parasitic species of the genera Anergates, Formicoxenus and Symmyrmica or in species like those of the genera Cardiocondyla, Technomyrmex and Ponera, which form small, scattered colonies, often with a tendency to lead a secluded or subterranean life. In the three parasitic genera the males are always wingless and resemble the females and workers in the structure of their bodies. The resemblance to the worker is very great

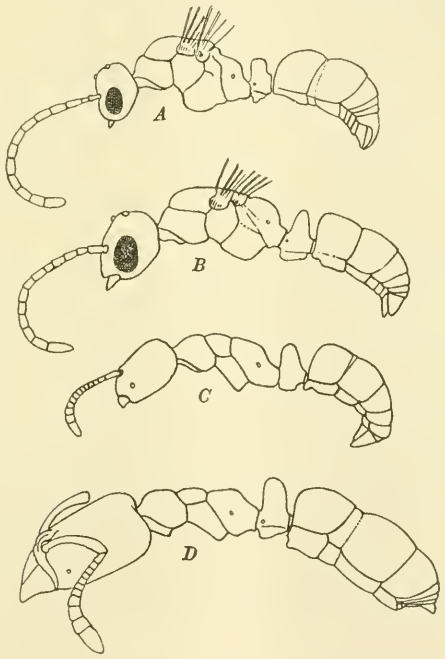


Fig. 8. *A*, winged male of Ponera coarctata in profile; *B*, winged male of P. eduardi; *C*, subergatomorphic male of the same species; *D*, ergatomorphic male of P. punctatissima (After Emery.)

in the case of Formicoxenus. In Cardiocondyla and Ponera we have a number of species whose males show a similar approximation to the worker and female type, and in one species of the latter genus, P. punctatissima, shown in the accompanying figure (Fig. 8*D*) the male is indistinguishable from the worker except in the structure of the genitalia. We have here, therefore, a true inversion of the male, so far as its secondary sexual characters are con-

cerned, apparently as an adaptation to ethological requirements, although the primary sexual characters have remained unaffected.

If it be true that the rudiments of the secondary sexual characters are set aside so early in the development of insects and remain uninfluenced by the internal secretions, we can understand why these characters exhibit no modification in cases of surgical castration and why the modifications induced by alimentary, nutricial and parasitic castration bear the aspect of inhibitions or retardations of growth. Normal imaginal development in insects, as is well known, depends on the amount of food accumulated during larval life and stored up in the fat-body. In insects surgically castrated during their younger stages there is nothing to hinder the accumulation of this reserve material, and all the imaginal characters, including the secondary sexual characters, are thereby enabled to develop normally and completely. But in insects that have been underfed or are infested with parasites the reserve materials are either prevented from accumulating or are consumed, so that the imago may have great difficulty in developing its imaginal characters. It is not surprising that under such conditions the secondary characters are more or less reduced or aborted, as we see in the forceps of parasitized Forficula males, the thoracic and cephalic horns of male Scarabæidæ, the mandibles of male Lucanidæ, the wings of female Lasii, and many of the other cases cited above. There is simply not enough nutriment to permit of the full growth of the characters under consideration. Their modification, therefore, is readily explained in insects as due to malnutrition and we are not compelled to invoke the internal secretions, or hormones, which play such an important and interesting rôle in the sexual physiology of vertebrates.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, E. L. MARK, DIRECTOR, No. 210

A COMPARISON OF THE REACTIONS OF A SPECIES OF SURFACE ISOPOD WITH THOSE OF A SUBTERRANEAN SPECIES

PART II

A. M. BANTA

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This paper is the second of a comparative study of the reactions of the surface isopod Asellus communis Say and its widely distributed subterranean relative Cæcidotea stygia Packard. The study was undertaken to determine in what ways and to what extent these animals differed physiologically and to learn why the one animal is a cave inhabitant while the other, its near relative living in the same region, rarely occurs in caves.

[1] Part I, on the reactions to light, has already been published in the JOURNAL OF EXPERIMENTAL ZOÖLOGY, vol. 8, no. 3, p. 243.

The work was done at the Museum of Comparative Zoölogy at Harvard College under the direction of Prof. G. H. Parker, to whom grateful acknowledgement is made.

I. EXPERIMENTS WITH MECHANICAL STIMULATION

1. With Bristles

The first method of testing the sensitiveness of the two animals to mechanical stimulation was by touching various parts of their bodies with delicate bristles. Six bristles ranging from 0.3 mm. in diameter to the finest camel's hair were employed at first, but it was found unnecessary to use so many and finally three were selected for use, a coarse pig bristle 0.3 mm. in diameter, a human hair and a fine camel's hair. The bending strain of the three bristles was 1.7 grams, .0025 gram, and .001 gram respectively. These will be referred to in future as bristles 1, 2, and 3. They were firmly fixed to the ends of slender glass rods by means of small rubber bands. About one centimeter of the bristle extended beyond the end of the rod.

The animals to be experimented with were placed each in a separate glass dish containing water to a depth of about two centimeters. Dishes with either ground-glass or wax-covered bottoms were used, since a smooth glass surface afforded no foothold for the animals and they were unable to move with certainty upon it. Since Cæcidotea normally lives in water a at temperature near 11°C., the water was kept at about this temperature during the experiments. Asellus, being normally subjected to a considerable range of temperatures, would probably not be much influenced by slight changes of heat and cold, but for the sake of uniformity it was kept and experimented upon at the same temperature as Cæcidotea.

One specimen of each species was tested at a time. The dishes containing the two individuals to be used were placed in a larger dish of water so that the temperature of the two animals would remain the same. A thermometer was kept between the two small dishes. From time to time cold water or bits of ice were added to

the water in the larger dish to keep the temperature from rising. When necessary the excess of water was removed.

The animals were given fifteen minutes or longer to become somewhat settled in their new quarters. When they had apparently begun to act and move about normally, the test was begun. First the largest bristle was used and the Asellus and Cæcidotea gently touched on various portions of the body and the sensitiveness as indicated by the animals' movements noted. No record was made until after several trials unless the reaction was unmistakable at once. First, for example, the Asellus was tested with one of the bristles upon the flagella of the antennæ, then the Cæcidotea was tested for the corresponding part. In like manner similar tests were made for other parts of the body. No special sequence was followed in testing the various portions of the body. Sometimes one portion was tested first and sometimes another But the corresponding parts of the two species were always tested one after the other.

The response to the various stimuli indicating corresponding grades of sensitiveness were designated by the following terms—extremely responsive, strongly responsive, fairly responsive, slightly responsive, and not responsive. An animal was considered *extremely* responsive if the movement was prompt and decidedly vigorous; *strongly* responsive if the response was slightly less prompt and vigorous than indicated for the extreme responses, but more vigorous than the normal movements of the animal *fairly* responsive when the reaction resembled the normal movements of the animal in rapidity and vigor; *slightly* responsive when there was any observable response less active or pronounced than the normal movements; and *not* responsive if no movements were observed after several attempts at stimulation. The following table will serve to illustrate the tests made and the manner of recording them.

This record (Table 1) is typical of the differences in sensitiveness to mechanical stimulation between the two species. It will be noted that whereas to bristle No. 1 the Asellus was very responsive, to bristle No. 3 it was scarcely responsive at all. With Cæcidotea the extreme responsiveness was as marked with the

small bristle. No. 3, as with No. 1. There are noticeable individual differences in both species, but in these tests the most responsive Asellus was less so than the least responsive Cæcidotea. The two individuals whose reactions are recorded in Table I represent for the two species about the average conditions as far as reactiveness to mechanical stimulation is concerned.

A series of ten pairs of individuals was tested and the results are summarized in Table II.

TABLE I

Reactions of Asellus communis, No. 2, ♂, length, 10.8 mm., and of Cæcidotea stygia, No. 2, ♂, length 10. 2 mm., to stimulation by bristles.

October 30, 1905. Temperature of Water, 11.4° C.

Bristle No. 1 (A pig bristle 0.3 mm. in diameter)

	Asellus communis	Cæcidotea stygia[2]
1. Flagella of the antennæ	*Slightly* responsive; moved the stimulated part occasionally	*Strongly* responsive; usually moved very quickly
2. Basal segments of the antennæ	*Strongly* responsive; moved the stimulated part or crawled	*Extremely* responsive; moved backward very quickly
3. Antennules	*Strongly* responsive; reached for bristle with antennæ and gnathopods	*Strongly* responsive; moved backward quickly
4. Top of head	*Strongly* responsive; moved appendages or crawled	*Extremely* responsive; with quick and vigorous movements
5. First free body segments	*Strongly* responsive; less vigorous reaction than when head was touched	*Extremely* responsive; similar movements but less vigorous than produced when head was stimulated
6. Other body segments	*Strongly* responsive; less vigorous reaction than when head or first segment was stimulated, but similar in character	*Extremely* responsive; with movements like those made when head or first segment was stimulated, but less vigorous
7. Legs	*Fairly* responsive; animal often moved	*Strongly* responsive; slightly less than when uropods were stimulated
8. Uropods	*Strongly* responsive; animal crawled quickly	*Strongly* responsive; usually crawled quickly

[2] This animal had recently undergone ecdysis and consequently was perhaps more than usually sensitive.

TABLE I—Continued

OCTOBER 30, 1905. TEMPERATURE OF WATER, 10.8° C.

Bristle No. 2 (A human hair)

	ASELLUS COMMUNIS	CÆCIDOTEA STYGIA
1. Flagella of the antennæ	*Slightly* responsive; moved antennæ or gnathopods	*Extremely* responsive; waved antennæ and crawled
2. Basal segments of the antennæ	*Strongly* responsive; quickly moved antennæ and gnathopods	*Extremely* responsive; waved antennæ and sometimes crawled backwards
3. Antennules	*Strongly* responsive; quickly moved antennæ and gnathopods	*Strongly* responsive; quickly moved antennæ and gnathopods
4. Top of head	*Slightly* responsive; moved antennæ	*Extremely* responsive; excited extremely vigorous movements which did not soon cease
5. First free body segments	*Slightly* responsive; moved antennæ or gnathopods	*Strongly* responsive; usually crawled
6. Other body segments	*Slightly* responsive; moved antennæ and gnathopods and finally crawled	*Strongly* responsive; usually crawled
7. Legs	*Slightly* responsive; moved leg to avoid stimulation or crawled	*Extremely* responsive; crawled quickly
8. Uropods	*Slightly* responsive; crawled occasionally	*Strongly* responsive; usually crawled

OCTOBER 30, 1905. TEMPERATURE OF WATER 11.2° C.

Bristle No. 3 (A small camel's hair)

	ASELLUS COMMUNIS	CÆCIDOTEA STYGIA[3]
1. Flagella of the antennæ	*Not* responsive; or only slightly so at times	*Extremely* responsive; moved violently at first touch
2. Basal segments of the antennæ	*Slightly* responsive; sometimes with movements of antennæ	*Extremely* responsive; moved violently at first touch
3. Antennules	*Slightly* responsive; moved antennæ or gnathopods often	*Extremely* responsive; crawled at once
4. Top of head	*Slightly* responsive; moved antennæ or gnathopods	*Extremely* responsive; crawled vigorously
5. First free body segment	*Slightly* responsive; moved antennæ or gnathopods	*Strongly* responsive; crawled, but less vigorously than when head was touched
6. Other body segments	*Not* responsive; or at best only very slightly so	*Strongly* responsive; crawled quickly
7. Legs	*Not* responsive	*Strongly* responsive; animal crawled
8. Uropods	*Slightly* responsive; moved after a time	*Extremely* responsive; crawled instantly and most vigorously

[3] A touch, however slight, produced a vigorous movement in nearly every case.

TABLE II

ASELLUS COMMUNIS

DEGREE OF RESPONSE	EXTREME			STRONG			FAIR			SLIGHT			NO		
BRISTLE	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1. Flagella of the antennæ				1	1		2			6	6	5	1	3	5
2. Basal segments of the antennæ	1			6	7	4	2	2	4	1	1	2			
3. Antennules				5	6	2	2	1	1	3	3	5			2
4. Top of head	2	1		8	2	3		4	3		3	4			
5. First free body segment	1	1		9	1	1		3	2		4	5		1	2
6. Other body segments				10	1			5	3		3	5		1	2
7. Legs	1			7			2	4	3		4	3		2	4
8. Uropods				6	2		4	1	1		5	4		2	5
Totals for each bristle	5	2	0	52	20	10	12	20	17	10	29	33	1	9	20
Totals for vigor of responses	7			82			49			72			30		

CÆCIDOTEA STYGIA

DEGREE OF RESPONSE	EXTREME			STRONG			FAIR			SLIGHT			NO		
BRISTLE	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1. Flagella of the antennæ	3	3	4	5	2	3		3	1	2	2	2			
2. Basal segments of the antennæ	7	5	4	3	3	5		2	1						
3. Antennules	4	1	3	4	6	4	1	2	1	1	1	2			
4. Top of head	6	6	5	4	3	4		1				1			
5. First free body segment	3	2		7	6	6		1	2		1	1			1
6. Other body segments	2	2		8	6	4		1	3		1	2			1
7. Legs	5	5	4	4	3	5	1	2				1			
8. Uropods	5	4	5	5	4	4		2	1						
Totals for each bristle	35	28	25	40	33	35	2	14	9	3	5	9	0	0	2
Totals for vigor of responses	88			108			25			17			2		

Table II is a summary of the vigor of responses made by ten individuals each Asellus of Cæcidotea. The tests were made on various parts of the body by bristles numbered 1, 2, 3,. No. 1 was a pig bristle 0.3 mm. in diameter, No. 2 a human hair, and No. 3 a fine camel's hair. In the first column to the left are designated the parts of the animals' body touched. In the successive triple columns to the right of the first column are indicated how many of the ten individuals experimented upon were *extremely* responsive when the various parts were stimulated by bristles 1, 2, 3, how many were *strongly* responsive, *fairly* responsive, *slightly* responsive and *not* responsive to stimulation upon the flagellum of the antenna by bristle No. 1, two were *fairly* responsive, six were *slightly* responsive, and one was *not* responsive at all. To stimulation upon the anntenules five Aseullus were *strongly* responsive to No. 1, six to No. 2 and two to No. 3, etc.

This summary indicates clearly that Cæcidotea is more sensitive to mechanical stimulation than Asellus is. Of the individual tests made upon the ten Cæcidotea 88 responses from the 240 trials indicated extreme sensitiveness and 108 indicated that the animal was strongly sensitive. The same test upon Asellus produced only 7 extreme responses indicating extreme sensitiveness and 82 strong responses. Of the whole number of tests with the three bristles, only 2 tests aroused no response in Cæcidotea, though 30 tests failed to produce reactions in Asellus. Bristle No. 1 produced 35 extreme responses with Cæcidotea and only 5 with Asellus. With bristle No. 3, 25 extreme responses were gotten from Cæcidotea and none from Asellus.

The responsiveness of Cæcidotea to the different bristles is very much the same, there being 35, 28 and 25 extreme responses to bristles Nos. 1, 2, and 3 respectively. With Asellus there is a very rapid falling off in responsiveness to the smaller bristles indicating a decided decrease in sensitiveness. The different bristles gave 5, 2 and 10 extreme responses and 52, 20 and 10 strong responses, respectively, indicating that with Asellus the predominating grade, of responsiveness to the coarsest bristle used is only of the grade, strongly sensitive, and that this responsiveness is much less pronounced with the finer bristles with which

only 20 and 10 such reactions were obtained. Hence it seems clear that Asellus is much the less responsive of the two species to this form of mechanical stimulation; that its responsiveness decreases rapidly with stimulation by the more delicate bristles; and that the threshold of stimulation is reached by the bristles used, whereas Cæcidotea is more responsive to such stimulation; in fact is nearly as responsive to stimulation by the smaller as by the larger bristles; and is extremely responsive beyond the threshold of stimulation for Asellus.

Asellus is more deliberate and less hasty in its reactions to mechanical stimulation than Cæcidotea is. This difference in the character of the reactions may influence one's judgment of the vigor of the reactions, so that the vigor of the response of Asellus is underestimated. Consequently a greater number of responses made by the Asellus possibly should be credited to the extreme column, than has been done. But if such an error should exist with reference to the extreme column for Asellus, it cannot affect the general result, as there can be no doubt of the diminution in number and vigor of the responses. Moreover, in many tests the actual lack of reaction in Asellus indicating lack of sensitiveness to more delicate stimulation is in strong contrast with the extreme sensitiveness of Cæcidotea.

The flagellum of the antennæ and the antennules are very delicate organs. Both are armed with many sensory hairs and might readily be thought highly sensitive to tactile stimulation. The flagella of the antennæ are relatively long and in both species, when the animals crawl, these organs extend in advance for a distance equal to more than half the length of the body. They appear to serve as important organs of touch. However, it appears that these organs in both species are relatively slightly sensitive to tactile stimulation of the sort employed. A remarkable difference exists between the sensitiveness of the flagella of the antennæ in the two; in Cæcidotea the flagella are only moderately sensitive, but in Asellus they are scarcely sensitive at all.

From the foregoing experiments the following conclusions may be drawn:

1. Asellus is decidedly less sensitive than Cæcidotea to mechanical stimulation by delicate bristles.

2. The responsiveness of Asellus decreases rapidly with stimulation by the more delicate bristles, while Cæcidotea was nearly as responsive to the finest as to the coarsest bristle used.

3. The threshold of stimulation for Asellus is much above that for Cæcidotea.

4. The antennules and flagella of the antennæ in both species are only slightly sensitive to mechanical stimulation.

5. The flagella of the antennæ are very much more sensitive in Cæcidotea than in Asellus, in which they are scarcely sensitive at all.

II. With Localized Currents of Water

A second kind of test for the sensitiveness of Asellus and Cæcidotea to mechanical stimulation was made by using localized currents of water. Fine glass tubes of various calibers were used through which delicate but constant currents of water were carefully directed upon the various parts of the animals. This afforded an easily controlable means of testing sensitiveness to mechanical stimulation.

The animals, as before, were placed in small wax-bottomed glass dishes containing water to a depth of 2 cm. These small dishes were put into a larger dish of water in which was a thermometer. The water was kept as near 11° as possible. A gallon bottle nearly filled with water was placed upon a support on the table so that the water level within the bottle was about 40 cm. above the level of the top of the table. Water was siphoned from this bottle through a rubber tube, into the free end of which was inserted a short glass tube drawn out to a fine point. The siphon flowed with a constant current and the rubber tube permitted the short glass end to be freely moved about, making it possible to direct the current wherever desired. A thermometer was kept suspended in the supply bottle. Water of the proper temperature

to keep the whole contents of the bottle near 11°C. was added from time to time in such amounts as to maintain a nearly constant level in the bottle. Tubes of four sizes were used. The bore of the largest was 118 μ in diameter and allowed a flow of water at the rate of 73 cc. per hour. This will be referred to as current No. 1. The next in size had a diameter of 90μ, and permitted a flow of 25 cc. per hour; its current will be designated as No.2. The third was 38μ in diameter and permitted a flow of 18 cc. per hour; its current will be callled No. 3. The smallest was 26μ in diameter and allowed a flow of 15 cc. her hour; its current is No. 4. In a few cases a fifth tube 12μ in diameter and allowing a flow of about 4 cc. per hour was used; current No. 5. The diameters of the tubes were only approximately obtained but the rates of flow of water through the tubes were readily and accurately determined and these were made the basis of selecting the currents of various strengths.

The individuals to be experimented upon were given at least fifteen minutes to become settled in the dishes before experimentation began. The corresponding parts of an individual of each species were tested in succession by a given current, but no regular sequence was followed in testing the various parts. After all the tests upon a given pair were made with one strength of current, the experiments were repeated with the weaker currents till all four currents had been used. In making these tests, the end of the tube which directed the current was always held under water and at 4 to 5 mm. from the part stimulated. Care was exercised to have the current of water flow squarely upon the part undergoing the test and so directed that other parts of the animal were not affected by it.

Records were kept after the same plan as was used for the tests with bristles (see p. 441), except that in the experiments with currents no records were made other than those of the vigor of the responses, for, even in the experiments with bristles this feature was finally found to be the only significant one. The following record, Table III, in which current 5, as well as the four usual ones, was used, will illustrate the results obtained from stimulation of this sort.

Both the individuals whose reactions are recorded in Table III were active and vigorous animals and the records shown are typical of the two species. It will be noted that Asellus was less sensitive than Cæcidotea to all the currents and scarcely sensitive at all to current No. 4, while Cæcidotea was extremely sensitive to the four currents and was only slightly less sensitive to the

TABLE III

Reactions of Asellus communis, No. 7, ♂, length 9 mm. and Cæcidotea stygia, No. 7, ♂, length 8.8 mm. to mechanical stimulation by small, locally directed currents of water. The first column ot the left indicates the parts stimulated. In the second to sixth columns are indicated the different currents used (Nos. 1 to 5) and the vigor of the reactions to these currents when directed upon the parts of the body indicated

ASELLUS COMMUNIS, NO. 7, ♂. 9 MM.
Temperature of Water, 12°C.

CURRENTS USED	No. 1[4]	No. 2	No. 3	No. 4	No. 5
1. Flagella of the antennæ.	Little	Little	Not	Not	Not
2. Basal segments of the antennæ.....	Extremely	Extremely	Strongly	Strongly	Not
3. Legs.....	Strongly	Strongly	Not	Not	Not
4. Top of head.....	Extremely	Extremely	Strongly	Strongly	Not
5. First free body segment	Strongly	Strongly	Fairly	Not	Not
6. Other body segments..	Strongly	Strongly	Strongly	Little	Not
7. Abdomen.....	Strongly	Strongly	Not	Not	Not
8. Uropods.....	Strongly	Strongly	Not	Not	Not

CÆCIDOTEA STYGIA, NO. 7, ♂, 8.8 MM.
Temperature of Water, 12°C.

CURRENTS USED	No. 1	No. 2	No. 3	No. 4	No. 5
1. Flagella of the antennæ	Not	Not	Not	Not	Not
2. Basal segments of the antennæ.....	Extremely	Extremely	Extremely	Extremely	Strongly
3. Legs.....	Extremely	Extremely	Extremely	Extremely	Not
4. Top of head	Extremely	Extremely	Extremely	Extremely	Strongly
5. First free body segment	Extremely	Extremely	Strongly	Strongly	Not
6. Other body segments...	Extremely	Extremely	Strongly	Strongly	Not
7. Abdomen.....	Extremely	Extremely	Extremely	Extremely	Not
8. Uropods.....	Extremely	Extremely	Strongly	Extremely	Not

[4]For a description of these currents, see page 448

weakest than to the strongest of them. With current No. 5, however, which was probably much below the threshold of stimulation for Asellus, Cæcidotea showed no sensitiveness except upon the head and the base of the antenna.

All the tests with currents 1 and 2 produced *extreme* responses Cædicotea except those directed upon the flagellum of the antenna. The same tests with Asellus produced *extreme* responses only upon the head and base of the antenna. Current 4 produced five extreme responses in the eight tests upon Cæcidotea, while but three tests aroused any response at all from Asellus. Current 5 aroused no response whatever from Asellus while Cæcidotea showed sensitiveness to stimulation by it upon the head and bases of the antennæ.

Ten pairs of Asellus and Cæcidotea were similarly experimented upon. Records of these experiments are summarized in Table IV.

Thus to stimulation upon the head by current No. 1, 7 Asellus were *extremely* responsive, 3 *strongly* responsive and none *fairly*, *slightly* or *not* responsive. To currents 1, 2, 3, and 4 applied upon the head a total of 16 were *extremely* responsive, 14 *strongly* responsive, 6 *fairly* responsive, 1 *slightly*, and 3 *not* responsive, etc.

This table shows that to mechanical stimulation by localized currents of water, Cæcidotea is decidedly more responsive than Asellus. Out of a total of 320 individual tests made upon 10 animals of each of these species, Cæcidotea gave 168 *extreme* responses and 51 failures to respond, while Asellus gave 61 *extreme* responses and 90 failures to respond.

The flagella of the antennæ are strangely unresponsive to this sort of stimulation and will receive special consideration elsewhere. If for the present they be left out of account, the number of tests followed by no responses in Cæcidotea is reduced to 15, though in Asellus it remains at 73, nearly five times as many.

The difference in sensitiveness is equally marked when the results of tests by the different currents are compared. Cæcidotea gave 59, 53, 36, and 20 *extreme* responses to currents 1, 2, 3, and 4, respectively, while Asellus gave 32, 23, 6, and 0 *extreme* responses to corresponding tests. With the weakest current (No.

TABLE IV

Summary of the reactions of ten Asellus and of ten Cæcidotea to localized currents of water. In the first column to the left are indicated the parts of the body against which the currents were directed. In the succeeding columns, each divided into four minor columns, are given the numbers of individuals out of a total of 10, EXTREMELY, STRONGLY, FAIRLY, SLIGHTLY, *and* NOT *responsive to stimulation upon the various parts by currents 1, 2, 3 and 4, respectively*

ASELLUS COMMUNIS

DEGREE OF RESPONSE	EXTREME				STRONG				FAIR				SLIGHT				NO			
CURRENTS USED	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1. Flagella of the antennæ					1				3	2	1		4	6	5	2	2	2	4	8
2. Basal segments of the antennæ	7	6	3		3	2	5	3		1	1	3		1	1					4
3. Top of head	7	7	2		3	2	5	4		1	3	2				1				3
4. First, free body segment	4	1			4	7	3		2	2	1				2	1			4	9
5. Other body segments					8	7			1	1	3		1	2	2				5	10
6. Abdomen	4	1			3	5			1	3	3		2	1	2				5	10
7. Legs	5	3			4	4	2			1	3		1	2	3				2	10
8. Uropods	5	5	1		2	2	4		2	2	1			1	2	1	1		2	9
	32	23	6	0	28	29	19	7	9	13	16	5	8	13	17	5	3	2	22	63
	61				83				43				43				90			

CÆCIDOTEA STYGIA

DEGREE OF RESPONSE	EXTREME				STRONG				FAIR				SLIGHT				NO			
CURRENTS USED	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1. Flagella of the antennæ									1				1	1	1		8	9	9	10
2. Basal segments of the antennæ	8	8	6	5	2		3	1		2		1			1	1				2
3. Top of head	10	9	9	5		1	1	3				2								
4. First free body segment	8	7	4	1	2	2	3	5		1	1	1			2					3
5. Other body segments	8	6			2	3	6	5		1	1	1			3	2				2
6. Abdomen	9	8	4	1			4	4	1	1	1	2		1	1					3
7. Legs	9	7	7	3		2	3	4	1	1		1								2
8. Uropods	7	8	6	5	3	2	3	2			1									3
	59	53	36	20	9	10	23	24	3	6	4	8	1	2	8	3	8	9	9	25
	168				66				21				14				51			

4) 20 *extreme* responses were given by Cæcidotea and only 25 of the tests; 10 of which were upon the flagellum of the antenna, failed to produce any reaction at all. With Asellus the same tests produced no *extreme* responses, while there were 63 failures to respond out of a total of 80 tests. Hence it appears that Cæcidotea is quite responsive to the weakest current used while Asellus is only slightly so.

The same relative lack of sensitiveness noted in the flagella of the antennæ, when stimulated by the bristles, was again noted with the currents. To stimulation by localized currents the flagella in both species are rather insensitive. This is especially true with Cæcidotea, whose flagella seemed less sensitive to this sort of stimulation than those of Asellus. To stimulation by bristles the reverse was true for in those experiments the flagella of the antennæ were more sensitive in Cæcidotea than in Asellus. A possible explanation of this discrepancy is that bristles tend to produce vibrations more than currents do and that the flagella are probably quite sensitive to frequent vibrations. This will be taken up again when the discussion of the reactions to sound is reached. The portions of the body of Asellus most sensitive to stimulation by currents are the head and the bases of the antennæ. They were almost the only parts responsive to the weakest currents. In Cæcidotea the maximum sensitiveness is less confined to the head and flagella of the antennæ than in Asellus, since it is shared somewhat by the uropods and legs. This point was well brought out by using current 5 with a number of pairs of individuals. Cæcidotea was found to be somewhat sensitive to this current upon the head, base of the antennæ, uropods and legs, but Asellus was seldom sensitive to it at all and never except occasionally upon the head and bases of the antennæ.

From these experiments upon the effects of mechanical stimulation by localized currents of water the following conclusions are drawn:

1. Cæcidotea is much more sensitive to water currents than Asellus.

2. The sensitiveness of Cæcidotea is only a little less marked

with the weakest than with the strongest of the four currents used, while the sensitiveness of Asellus almost disappears within the same range of stimulation.

3. The threshold of stimulation for Cæcidotea is considerably below that for Asellus.

4. The flagella of the antennæ in both species are only slightly sensitive; contrary to what is usually true, to water c rrents this organ is more sensitive in Asellus than in Cæcidotea.

5. The most sensitive parts of the body in each species are the head and bases of the antennæ, but the uropods and legs of Cæcidotea are only slightly less sensitive than such other parts.

3. *With the Concussion Produced by a Falling Solid Body Striking upon a Surface of Wood*

A third method of testing the relative sensitiveness of Asellus and Cæcidotea to mechanical stimulation was by trying the effect of a mechanical vibration produced by the concussion of a falling solid body upon a surface of wood. A steel ball and a lead shot were used. The steel ball weighed 3.505 grams; the shot 0.542 grams. At first only the steel ball was used and it was dropped through a distance of from 50 cm. to 2 cm. Since the threshold of stimulation was not reached for either species by the concussion produced by the steel ball, the lead shot was used in an endeavor to get a weaker stimulus. The momenta of the two falling bodies were calculated,[5] and the falling distances chosen so that the concussions produced by the two bodies would form a graded series.

The metal balls were dropped upon the flat, smooth surface of a pine board 30 cm. long, 19 cm. broad and 1.2 cm. thick. The balls were dropped from between the thumb and forefinger at various levels, as determined by a meter stick, and made to fall at a particular place on the board. The isopod to be experimented upon was put into a waxed-bottomed stender dish 6 cm. in diame-

[5] The effect of the friction of the air and the difference in the hardness and density of the two bodies were not taken into account.

ter. The dish was placed near the center of the board and 8 cm. from the place where the ball struck the board. In order to observe slight movements of Cæcidotea during the tests, these animals, which are white, were placed in a dish the bottom of which was black. For a similar reason the rather dark Asellus was experimented upon in a dish having a whitish bottom. In every case the animals were allowed to remain from 15 minutes to an hour in the dish to become accustomed to the new surroundings before the experiments were begun and no animal was experimented with until it had apparently begun to act and move normally after its transference.

In these tests the following grades of stimuli were used. The steel ball was dropped from 50 cm., 20 cm., 10 cm., 5 cm., and 2 cm., and had the following momenta at the instant of striking the board: 1097, 694, 491, 347, and 219 C. G. S. units.[6]

In using the lead shot for the lower range of stimuli, it was deemed advisable as a check to test the reactions of the animal to vibrations produced by both the steel ball and the lead shot when they collided with the board with the same momentum. Hence the shot was dropped 83.6 cm. and the ball 2 cm., so that in each case the bodies struck the board with a momentum of 219 C. D. S. units. The shot was used also from 50, 30, 20, 10 and 5 cm., producing momenta of 169, 131, 107, 76 and 54 C. G. S. units. The whole series of momenta used then was (1) 1097, (2) 694, (3) 491, (4) 347, (5) 219 (steel ball), (6) 219 (shot), (7) 169, (8) 131, (9) 107, (10) 76 and (11) 54 C. G. S. units. These will be referred to in future as momenta of grades 1 to 11 respectively. In testing an animal the greatest momentum was first used for 20 trials. After each individual trial the result was recorded and the animal allowed to come to rest again, if it had responded to the stimulus. If the animal seemed erratic in any way, subsequent sets of 20 tests were made till constant results were obtained. After com-

[6] momentum = m v

$v = \sqrt{2sg}$

$\therefore$ momentum $= m\sqrt{2sg}$

m = mass, s = distance through which the body falls, v = velocity, and g = gravity.

pleting the tests with one momentum for an Asellus, the dish containing the animal was gently removed from the table so as to be beyond the influence of the vibrations, and the experiments were repeated on a Cæcidotea. This method of procedure was followed for each pair of animals through all the eleven momenta used.

The kinds of reactions were designed by numbers as follows:

0, no reaction.

1, slight movements of antennæ or other appendages.

2, more extended movements of antennæ or other appendages.

3, movements of appendages and bending anterior end of body.

4, same as "2" or "3" followed by the animal's crawling.

5, crawling at once.

For each test the number corresponding to the reaction was recorded. Table V shows one of these records complete.

Table V indicates that the responsiveness of this Asellus was most pronounced to the vibrations produced by the falling bodies striking the wood with 1097 and 694 C. G. S. units of momentum, and that there was a rapid decrease as the lower momenta were reached. With Cæcidotea the reactions were most pronounced to the higher momenta used and a rapid decrease in responsiveness occurred with momenta below grade 4, but the responsiveness is not entirely lost even with momenta of grade 11, only 54 C. G. S. units. Cæcidotea responded not only more often but with a greater vigor of reactions to all the grades of stimuli. These two individuals are typical of the reaction of the two species as Table VI will show.

From Table VI Asellus is shown to be less reactive than Cæcidotea to this sort of stimulation. This difference appears not only in the number of the responses, but in the vigor of the responses, and in the range of responsiveness, for Asellus is not sensitive at all to such stimulation when the momentum is less than 169 units, while Cæcidotea appears somewhat responsive to as low a momentum as 54 units. Asellus gave the following aver-

TABLE V

ASELLUS COMMUNIS NO. 1, ♀, 9 MM. LONG

BODY USED	STEEL BALL					LEAD SHOT					
Distance of fall in cm	50	20	10	5	2	83.6	50	30	20	10	5
Momenta in C. G. S. units	1097	694	491	347	219	219	169	131	107	76	54
Grades of momenta	1	2	3	4	5	6	7	8	9	10	11
Reactions	0	1	0	0	0	0	0				
	1	2	0	0	0	0	0				
	3	5	1	0	0	0	0				
	2	4	0	0	0	0	0				
	0	2	0	0	0	0	0				
	0	0	0	0	0	0	0				
	3	0	0	0	0	0	0				
	0	0	0	0	0	0	0				
	0	0	1	0	0	0	0				
	1	0	1	0	0	0	0				
	2	2	0	0	2	0	0				
	3	3	0	0	0	1	0				
	4	4	0	2	0	0	0				
	2	0	0	1	0	0	0				
	2	1	1	0	0	0	0				
	1	1	0	0	0	0	0				
	3	4	0	0	0	0	0				
	5	3	0	0	0	0	0				
	1	0	0	1	0	0	2				
	0	4	0	4	0	0	0				
Number of responses in 20 trials	14	13	4	4	1	1	1				
Sum of responses	33	36	4	8	2	1	2				
Average vigor of responses	24	28	1	2	2	1	2				

TABLE V

CÆCIDOTEA STYGIA, NO. 1, ♂, 10 MM. LONG

BODY USED	STEEL BALL					LEAD SHOT					
Distance of fall in cm	50	20	10	5	2	83.6	50	30	20	10	5
Momenta in C. G. S. units	1097	694	491	347	219	219	169	131	107	76	54
Grades of momenta	1	2	3	4	5	6	7	8	9	10	11
Reactions	5	2	5	5	0	0	1	5	3	0	0
	5	5	4	4	0	0	0	0	3	0	0
	4	5	5	4	5	1	0	0	0	0	0
	5	1	1	2	0	4	4	0	0	0	0
	4	5	3	5	5	0	1	0	0	0	0
	2	1	2	4	0	0	0	0	0	0	0
	3	5	2	0	0	0	0	0	0	1	0
	5	5	5	4	0	0	0	0	0	0	0
	5	3	5	3	0	4	0	0	1	0	4
	3	3	4	5	1	2	0	5	1	1	0
	2	5	5	0	0	2	0	3	0	0	0
	4	4	2	3	0	4	0	0	1	1	0
	2	5	1	5	0	1	0	0	0	0	0
	0	2	5	4	1	0	4	0	0	0	0
	5	5	5	2	0	0	0	0	1	0	0
	5	4	5	5	2	1	1	0	0	0	0
	5	5	2	0	1	0	0	0	0	0	0
	3	1	2	4	1	0	0	3	0	0	0
	5	0	5	3	0	0	0	0	4	0	0
	4	1	1	4	4	1	0	4	0	1	0
Number of responses in 20 trials	19	19	20	17	8	8	5	5	7	4	1
Sum of responses	76	67	69	66	20	19	11	20	14	4	4
Average vigor of responses	4	3.53	3.45	3.88	2.5	2.37	2.2	4	2	1	4

TABLE VI

Summary of the reactions of six Asellus communis and six Cœcidotea stygia to mechanical vibrations produced by the concussions of bodies falling with various momenta. The body used in producing the concussion, the distance of its fall, its momentum at the time of producing the concussion, and the grade of its momentum, are indicated in successive horizontal columns at the top of the table. For each individual tested are given in italics the total number of reactions obtained out of 20 trials for each momentum used, and the sum of these reactions on the basis of their vigor. Following the summaries for the six pairs of individuals are given in the last four horizontal columns the total number of responses, the average number of reactions out of 20 trials, the average sum of the reactions for each 20 trials, and the average vigor of the reactions for each momentum used. For example, Asellus No. 1 gave 14, 13, 4, 4, 1, 1, 1, 0, 0, 0, and 0 reactions to the different momenta used, and the sums of the vigor of these reactions were 33, 36, 4, 8, 2, 1, 2, 0, 0, 0, and 0

ASELLUS

BODY USED		STEEL BALL					SHOT					
Distance of fall in cm		50	20	10	5	2	83.6	50	30	30	10	5
Momenta, in C. G. S. units		1097	694	471	347	219	219	169	131	107	76	54
Grades of momenta		1	2	3	4	5	6	7	8	9	10	11
No. 1, ♀, 9 mm. long	No. of responses	*14*	*13*	*4*	*4*	*1*	*1*	*1*				
	Vigor of responses	33	36	4	8	2	1	2				
No. 2, ♀, 8.5 mm. long	No. of responses	*16*	*16*	*11*	*4*	*1*	*2*	*0*				
	Vigor of responses	27	21	34	7	1	2	0				
No. 3, ♀, 9.4 mm. long	No. of responses	*9*	*5*	*6*	*3*	*0*	*0*	*0*				
	Vigor of responses	19	12	7	3	0	0	0				
No. 4, ♀, 9 mm. long	No of responses	*5*	*4*	*6*	*3*	*1*	*1*	*0*	No reac tion s			
	Vigor of responses	6	4	6	2	1	5	0				
No. 5, ♀, 6 mm. long	No. of responses	*5*	*4*	*4*	*4*	*2*	*1*	*0*				
	Vigor of responses	5	8	8	14	7	1	0				
No. 6, ♀, 10 mm. long	No. of responses	*18*	*10*	*7*	*0*	*0*	*2*	*0*				
	Vigor of responses	18	23	7	0	0	2	0				
Total number of responses		67	52	38	18	5	7	1				
Average number of responses to 20 trials		$11\frac{1}{6}$	$8\frac{2}{3}$	$6\frac{1}{3}$	3	$\frac{5}{6}$	$1\frac{1}{6}$	$1\frac{1}{6}$				
Average sum of the responses to 20 trials		18	$17\frac{1}{3}$	11	$5\frac{4}{3}$	$1\frac{1}{6}$	$1\frac{5}{6}$	$\frac{1}{3}$				
Average vigor of responses		1.61	2	1.74	1.88	2.2	1.57	2				

Average vigor of responses to whatever stimulus, 1.79.

TABLE VI– Continued

CÆCIDOTEA

BODY USED		STEEL BALL				SHOT						
Distance of fall in cm.		50	20	10	5	2	83.6	50	30	20	10	5
Momenta, in C.G.S. units		1097	694	471	347	219	219	169	131	107	76	4
Grades of momenta		1	2	3	4	5	6	7	8	9	10	11
No. 1, ♂, 10 mm. long	No. of responses	19	19	20	17	7	8	5	4	7	4	1
	Vigor of responses	75	67	69	66	20	29	11	16	14	4	4
No. 2, ♀, 7.4 mm. long	No. of responses	20	20	20	19	5	6	2	2	3	2	2
	Vigor of responses	94	75	78	72	14	24	9	8	9	8	2
No. 3, ♀, 7 mm. long	No. of responses	12	5	3	4	5	5	1	1	1	1	0
	Vigor of responses	41	11	6	18	23	16	3	1	1	1	0
No. 4, ♀, 7 mm. long	No. of responses	18	14	12	10	4	2	1	1	2	2	3
	Vigor of responses	55	37	22	21	12	8	4	1	6	7	6
No. 5, ♀, 5 mm. long	No. of responses	16	16	14	11	7	8	10	9	3	3	1
	Vigor of responses	63	67	47	42	17	22	23	20	6	7	3
No. 6, ♀, 7 mm. long	No. of responses	18	15	11	10	6	5	4	2	3	1	0
	Vigor of responses	64	55	36	28	26	21	16	17	11	3	0
Total number of responses		103	89	80	71	34	34	23	19	19	13	7
Average number of responses to 20 trials		$17\frac{1}{6}$	$14\frac{5}{6}$	$13\frac{1}{3}$	$11\frac{5}{6}$	$5\frac{2}{3}$	$5\frac{2}{3}$	$3\frac{5}{6}$	$3\frac{1}{6}$	$3\frac{1}{6}$	$2\frac{1}{6}$	$1\frac{1}{6}$
Average sum of the responses to 20 trials		$65\frac{1}{2}$	52	43	$41\frac{1}{6}$	$18\frac{3}{4}$	$19\frac{1}{4}$	11	$10\frac{1}{2}$	$7\frac{5}{6}$	5	$2\frac{1}{2}$
Average vigor of responses		3.8	3.53	3.23	3.48	3.29	3.41	2.87	3.31	2.47	2.3	2.14

Average vigor of responses to whatever stimulus, 3.37

age number of responses out of 20 trials to stimulations of grades 1 to 7, $11\frac{1}{6}$, $8\frac{2}{3}$, $6\frac{1}{3}$, $3\frac{5}{6}$, $1\frac{1}{6}$, and $\frac{1}{6}$. To the same grades of stimuli Cæcidotea gave the following average number of reactions: $17\frac{1}{6}$, $14\frac{5}{6}$, $13\frac{1}{3}$, $11\frac{5}{6}$, $5\frac{2}{3}$, $5\frac{2}{3}$, and $3\frac{5}{6}$, while the reactiveness continued to stimuli of grades 8, 9, 10, and 11 with the following average number of responses: 3, $\frac{1}{6}$, $2\frac{1}{6}$, $2\frac{1}{6}$, and $1\frac{1}{6}$. The averages for the vigor of the responses of Asellus for the grades of stimuli 1 to 7 were of the grades of response 1.61, 2, 1.74, 1.88, 2.2, 1.57, and 2. The corresponding averages for Cæcidotea were 3.8, 3.5, 3.23, 3.48, 3.29, 3.41, and 2.87, while to the lowest grades of stimulation to which Asellus did not respond the averages of Cæcidotea's responses were 3.31, 2.47, 2.3, and 2.14. The average on the basis of vigor of response for the whole number of reactions obtained from Asellus

is 1.79; the same average for Cæcidotea is 3.37. Asellus, out of 120 trials, 20 upon each of six animals gave but one reaction to stimulation by grade 7 (169 units of momentum) and no reactions were gotten from stimulation below this grade. Cæcidotea continued to respond to the lower grades and to some extent even to the lowest grade used (54 units of momentum). The close agreement of results obtained by grades 5 and 6, in which different bodies strike with the same momentum removes any reasonable doubt as to the justification of considering the effectiveness of the stimulation to be dependent upon the momentum.

As already noted it sometimes occurs that animals upon which tests were being made were moving at the time of the test. Under such circumstances the movements of the animals were often arrested. An arrest of movements due to a stimulus is of course as definite a reaction as the inciting to movement by a stimulus. Such arrests of movements were recorded with the other reactions.

One of the most delicate reactions was a twitching of the flagellum of the antenna or a stroking of the antenna by the gnathopod. This reaction suggested the possibility that the flagellum is particularly sensitive to such mechanical stimulation.

The results of the foregoing experiments may be summarized as follows:

1. Falling bodies having the same momenta produce virtually the same reactions. These become more numerous as well as more vigorous with increase of momentum.

2. Asellus responds much less often than Cæcidotea to such stimuli of whatever grade.

3. Asellus responds less vigorously than Cæcidotea to such stimuli; the average of the vigor of responses for the whole number of responses for Asellus was 1.79 and for Cæcidotea 3.37.

4. The threshold of stimulation for Asellus was found to be near that of grade 5 and 6 (219 units of momentum), while the threshold for Cæcidotea was approached only with the lowest stimulation used, grade 11 (54 units of momentum), a grade only $\frac{1}{4}$ as high.

4. *With Vibrations, 100 per Second*

A fourth method of testing the relative responsiveness of these two species to mechanical stimulation was by means of an electric tuning fork whose rate was 100 complete vibrations per second. The animals, as in previous experiments were placed in small stentor dishes, the Asellus over a light-colored bottom and the Cæcidotea over a black one. The stentor dish containing the animal was placed upon a heavy block of hard pine wood 41 cm. long, 22 cm. broad and 11 cm. thick, with planed surfaces. The dish was firmly fixed upon the block near one end by being clamped between three nails driven partly into the block. The block rested upon a pile of crumpled paper two inches thick placed upon a table whose legs rested upon crumpled paper. Close by the first table was a second one likewise supported on paper, and upon this table lay the fork, also on a thick pad of paper. The piles of paper prevented any perceptible transmission of the vibrations (other than when desired) from the fork to the stentor dish in which the animal was confined. The fork was driven by a Columbia No. 6 dry cell and a simple key was used to make and break the circuit as desired. Two cells were used alternately to avoid the effects of running down.

The manner of experimentation was as follows: The animal to be tested was allowed to remain in the stentor dish in position for a sufficient length of time to become thoroughly settled, after which the fork was set in vibration and slipped over its support of paper (upon which rested a smooth sheet of glass) until its base came into firm contact with the end of the block on which the stentor dish rested. After a momentary contact the fork was withdrawn and a record of the response of the animal, if any was made. The test was repeated after about ten seconds or more. Twenty of these tests were made upon each individual, first as Asellus then a Cæcidotea, until 15 pairs of individuals had been tested. The responses were ranked, as in the experiments on the effect of stimulation by the concussions of falling balls (see p. 453), though it was found expedient to add another class. Sometimes the animals moved just at the instant before

the stimulus was applied and in such cases often an arrest of movement occurred. This was indicated by an "A." Table VII shows the detailed records of the fifteen pairs of individuals tested in this manner.

An examination of Table VII discloses the fact that in every case where a pair of the two species were subjected to test, the Cæcidotea was the more responsive of the two. Asellus made on an average for the 15 individuals tested 6.8 responses in 20 trials, with an average vigor of 1.39, while the 15 Cæcidotea averaged 11.8 responses in the same number of trials, with an average vigor of 3.02. Hence the number of responses made by Cæcidotes was nearly twice as large as the number made by Asellus and at the same time they were on the average considerably more than twice as vigorous.

Very often, particularly with Asellus, the animal responded by trembling or quivering movements of the flagellum of the antenna or a quick stroking of the antenna with the gnathopod. Such reactions occurred often with this sort of stimulation as well as with the stimulation from the concussions of falling balls, but to stimulation by bristles or locally applied currents of water it was scarcely ever noted. These results suggest that the response to mechanical vibrations may be more or less localized in the flagellum of the antenna.

The following statements summarize these results:

1. Asellus is less sensitive to such vibrations than Cæcidotea.

2. Asellus responds to such stimulation scarcely more than half as often as Cæcidotea.

3. Asellus responds to such stimulation less than half as vigorously as Cæcidotea.

4. Arrest of movements is often caused by this form of stimulation.

5. The fact that the antennæ are so often whipped about and rubbed by the gnathopod during such stimulation suggests that they are more sensitive to this stimulus than other parts of the body although to pure tactile stimulation they are scarcely at all sensitive.

5. *General Summary of Experiments with Mechanical Stimulation*

To all kinds of mechanical stimulation, whether pure tactile stimulation or mechanical vibrations, Cæcidotea is decidedly more responsive than Asellus. This becomes evident both from examining the frequency of response and the vigor of response, Cæcidotea responding more often and more vigorously than Asellus. The difference in vigor of response is particularly noticeable toward the stimuli of lower intensity to which Asellus responds only very slightly, if at all; but Cæcidotea, as long as the stimulus produces any response at all, continues to respond vigorously.

The flagella of the antennæ of both species were surprisingly unresponsive to pure tactile stimulation by contact with bristles or localized currents of water, but they seemed to be extremely sensitive to vibrations such as those procuced by the tuning fork or by the concussion of a falling body. While the latter was not actually demonstrated, it was strongly indicated by the different behavior of the animals under the two sorts of stimulation—pure tactile and mechanical vibrations. When the flagellum of the antenna did respond to tactile stimulation the response was usually accompanied, especially in Asellus, by a trembling or jerking motion and a withdrawal of the stimulated part or a stroking or rubbing of the whole antenna with the gnathopod. Such a response occurred very commonly to stimulation by concussion and by the tuning fork. With the concussions the stimulus was only momentary and the response was of short duration, but quite like the response to the vibrations of the fork, except that it was not carried so far. To the vibrations of the fork the response was quite often only the above-mentioned movements of the antennæ and gnathopods. If the stimulus was continued without break, however, these movements were usually followed by the animals' crawling. In short, the responses to stimulation of the animal as a whole by mechanical vibrations are the same as the responses to local stimulation of the flagella of the antennæ by pure tactile stimulations. In the latter case the stimulation was local, and

TABLE VII, Part 1

Part 1 shows the reactions of the 15 Asellus communis to stimulation by vibrations at the rate of 100 per second produced by an electric tuning fork. Part 2 shows the same for the 15 Cœcidotea stygia under similar conditions. The responses of each individual are indicated in a separate column. The characteristics used in indicating the reactions refer to the various grades of responses (see p.).

Following the detailed responses as indicated for each individual, are given in successive horizontal columns, the number of responses gotten from each animal in 20 trials, the sum of the vigor of these responses, and the average vigor of the responses for each individual tested. In each part of the table at the lower right hand corners are given the average number of responses out of 20 trials for each of the species, the average sum of the vigor of the responses to 20 trials for each species and the average vigor of the response for each species.

	ASELLUS COMMUNIS															
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	NO. 9	NO. 10	NO. 11	NO. 12	NO. 13	NO. 14	NO. 15	AVER-
	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	AGES
Responses.....	6.6 MM.	11 MM.	9 MM.	9.4 MM.	7.2 MM.	7 MM.	9.5 MM.	7 MM.	6.5 MM.	10 MM.	8.5 MM.	8.5 MM.	6.5 MM.	6.3 MM.	7.5 MM.	
	3	0	0	0	0	0	3	2	0	1	0	0	0	0	1	
	0	1	0	0	0	0	5	0	0	1	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	
	3	2	1	2	0	1	2	1	0	0	0	0	0	0	0	
	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	
	4	2	0	0	0	1	1	0	0	0	1	1	1	1	1	
	0	0	0	4	0	0	1	1	0	0	0	1	1	0	0	
	0	2	0	3	0	0	0	1	0	0	0	0	1	0	0	
	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	

	3	1	0	0	0	0	1	0	1	0	1	0	0	1	0	
	5	1	0	3	0	0	0	0	1	0	1	1	0	0	0	
	0	1	0	3	0	1	4	0	0	1	0	0	0	0	0	
	0	0	0	4	0	0	2	1	0	0	0	1	1	0	0	
	3	2	0	0	0	1	2	1	0	0	0	1	0	0	0	
	4	1	0	3	0	1	0	0	1	2	0	1	0	0	0	
	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	
	2	1	0	0	1	0	1	1	0	0	0	1	0	0	0	
	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	
	0	1	0	0	0	1	0	1	0	2	0	0	1	0	1	
	0	1	0	2	0	1	1	0	0	1	0	0	0	0	0	
Total number of responses	9	14	1	8	1	8	11	9	4	8	3	11	6	5	4	6.8
Sum of vigor of responses	28	18	1	24	1	8	23	10	4	10	3	11	6	5	4	10.4
Average vigor of responses	3.11	1.28	1	3	1	1	2.09	1.11	1	1.25	1	1	1	1	1	1.39

TABLE VII, Part 2

CÆCIDOTEA STYGIA

	No. 1 ♀ 7 mm.	No. 2 ♂ 7.5 mm.	No. 3 ♂ 7.5 mm.	No. 4 ♂ 7.5 mm.	No. 5 ♀ 6.5 mm.	No. 6 ♀ 7 mm.	No. 7 ♂ 10 mm.	No. 8 ♀ 6 mm.	No. 9 ♂ 7 mm.	No. 10 ♀ 8 mm.	No. 11 ♂ 6 mm.	No. 12 ♀ 5.7 mm.	No. 13 ♀ 5 mm.	No. 14 ♀ 6.3 mm.	No. 15 ♀ 6 mm.	Averages
Responses	1	5	0	3	5	0	0	5	0	0	0	1	5	3	0	
	2	0	0	0	0	1	0	5	0	0	5	0	5	5	A	
	5	5	1	5	2	4	2	4	0	0	5	2	0	0	5	
	0	0	3	0	2	0	0	1	5	5	5	0	2	0	1	
	3	3	0	5	0	4	3	0	2	0	3	0	5	2	0	
	0	3	0	0	1	1	1	5	1	2	1	0	5	2	1	
	0	3	5	5	1	0	0	0	0	1	0	1	3	0	0	
	5	3	5	5	2	0	0	0	3	5	0	2	5	0	3	
	4	3	0	0	3	3	1	0	3	2	1	5	0	5	0	
	0	3	0	5	0	4	1	2	0	0	5	0	5	A	3	
	1	2	0	5	3	3	1	0	1	1	5	1	5	0	1	
	2	0	5	0	0	3	5	0	0	3	3	0	3	0	0	
	5	4	1	5	5	0	0	0	2	0	A	0	5	0	0	
	0	0	0	0	1	2	0	0	1	3	5	3	5	0	1	
	0	3	0	5	2	4	0	0	1	2	5	2	0	5	3	
	0	1	0	2	1	0	2	0	5	0	0	0	A	5	0	
	0	1	4	1	1	3	5	0	4	0	5	0	3	4	3	
	5	3	0	2	2	0	0	0	0	0	5	1	5	3	0	
	5	2	0	0	1	0	3	0	0	5	A	0	0	5	**3**	
	1	3	1	0	1	2	0	0	5	5	2	0	0	0	0	
Total number of responses	12	16	8	12	16	12	10	6	12	11	16	9	15	11	11	11.8
Sum of vigor of responses	39	47	25	48	33	34	24	22	33	34	55	18	61	39	24	35.73
Average vigor of responses	3.25	2.94	3.12	4	2.06	2.83	2.4	3.66	2.75	3.09	3.44	2	4.07	3.55	2.18	3.02

the response was local; in the former the stimulation was general but the response was local. Hence it would seem that inasmuch as the first and most common response to a general stimulation of the animal by mechanical vibration is in movements of the antennæ, accompanied by movement of the gnathopod, which response is the same as that produced by local stimulation of the antennæ by pure tactile stimulation, that the antennæ are particularly sensitive to vibrations, and that we here have a case of specialization in which the antennæ, though only slightly sensitive to pure tactile stimulation, are decidedly sensitive, and, in fact, the parts most sensitive to mechanical vibrations.

It was noticed repeatedly that Asellus and Cæcidotea were very sensitive to any movement in the water. This sensitiveness was much the more marked in Cæcidotea. Even dipping a small camel's hair brush or the end of a pencil into the water would often rouse a Cæcidotea to sudden movement, notwithstanding that it might be 40 to 50 cm. away from the point where the disturbance started. A few times during experimentation with Cæcidotea when I accidentally breathed upon the surface of the water, the animals responded vigorously. This acute sensitiveness to a disturbance in the water was noted, to, in collecting Cæidotea in the caves, where slight and unusual movements in the water at one side of a pool would arouse to active movements every Cæcicidotea within a radius of 2 or 3 meters, the animals generally leaving the borders of the pools and moving toward the deeper water where they soon became concealed under the edges of stones or in small depressions in the bottom.

This greater sensitiveness of Cæcidotea to mechanical stimulation of whatever sort and its relatively slight response to light is a good illustration of the principle of compensation in special senses.

II. EXPERIMENTS WITH THE ANIMALS IN CURRENTS OF WATER

A special trough was constructed for experiments on the effects of currents of water on Asellus and Cæcidotea. Two thin trips of glass, 46 cm. long by 2 cm. wide, were cemented in vertical par-

allel planes, 2.3 cm. apart, upon a base of slate 46 cm. long by 6 cm. wide. The slate afforded a fairly good foothold for the animals which crawl, but do not swim. This base of sufficient width to prevent an easy overturning of the trough. The upper end of the trough, into which the water flowed, was closed by a piece of tin to within 4 mm. of the top; the lower end was closed also by a piece of tin which, however, rose only to within 1 cm. of the top. Closely fitting partitions of copper gauze 16 meshes to the centimeter were used to confine the animals within the more nearly central part of the trough, where the current was uniform. Across the tank over each of these partitions was laid a piece of glass to prevent the animals from crawling over the partition.

The trough when in use was supported upon a small stand. A glass jar 31 cm. high was placed close to the upper end of the tank and under a faucet. The jar was kept filled to overflowing with water during the experiments. Siphons of various sizes, depending upon the strengh of current desired within the trough were used to conduct the wate from the jar to the trough. The gauze partitions were placed at 10.5 cm. from the upper end and 3.5 cm. from the lower end, this making the functional part of the trough, within which the animals were confined, 32 cm. long.

The siphon used introduced the water near the extreme upper end of the trough and below the level of the water. There were considerable irregularities in the upper portion of the current. In order to break up these irregularities and reduce the current to uniformity, three partitions of wire netting, 5 meshes to the centimeter, were interposed at intervals of about 2 cm. between the place where the siphon discharged into the trough and the upper partition of copper gauze. This arrangement secured a practically uniform current throughout that portion of the trough in which the animals were placed.

During some of the experiments one end of the tank was darkened while the other end was illuminated by direct sunlight, diffuse day light or by a 6-glower Nernst lamp. The part of the trough darkened was covered with a closely fitted black cloth; when the upper end was covered the cloth was held about the siphon by a stout rubber band, and when the lower end was dark-

ened the cloth was formed into a rude hanging tube through which the water escaped. While this arrangement certainly did not make the darkened end light-proof, it at any rate cut off nearly all the light except that which entered from the light part of the trough. Doubtless a great deal did so enter, but under all circumstances there was a strong contrast between the illuminated part of the trough and the rest as judged by the eye.

Ten animals were found most satisfactory to experiment with at a time, but occasionally as few as five or as many as twenty were used. In general, currents of two strenghts were used: 435 centimeters per minute and 140 centimeters per minute. Currents stronger than 435 cm. per minute swept the animals, especially the Asellus, down the trough and currents weaker than 140 cm. seemed to produce no decided results.

1. *Asellus*

When, after having become quiet, Asellus was subjected to a current of water in the apparatus described, it usually responded very quickly by crawling against the current. Individuals which went down-stream soon turned back from the partition and crawled up-stream. Individuals which reached the upper end did not usually turn back. Hence the rheotaxis was very marked.

The animals, when first subjected to a current, crept rapidly for a time. In doing so they did not adhere well to the slate bottom of the trough, and many were swept off their feet and carried all or part way towards the lower end of the trough. Those swept down ordinarily persisted in crawling up-stream again, and after a time, when they had come to crawl less rapidly, fewer were carried back by the current, and they became collected at the upper end of the trough.

Asellus tends somewhat to remain on the copper gauze partitions at the ends of the trough. The great amount of moving back and forth in the trough especially during the early part of an experiment is sufficient warrant that this is not a very disturbing factor, however.

The maximum response as indicated by the number of indi-

viduals which collected at the upper end of the trough was attained in from five minutes to four and a half hours after the experiment began, but on the average it occurred in about 1.4 hours. The persistence of the response was extremely variable; in some cases the animals remained at the upper end of the trough for only about a quarter of an hour, while in one experiment (No. 16) they stayed continuously for two days, after which they gradually left the upper end of the trough. Commonly, however, the rheotactic response did not persist more than two or three hours, and in cases in which the experiment was run over night, the response usually became decidedly reduced before the next morning.

Table VIII shows the results of the characteristic experiments of this series.

2. *Cæcidotea*

Cæcidotea, when first subjected to a current of water, moved about usually with little reference to its direction, though it sometimes reacted almost at once, crawling towards the upper end of the trough. Cæcidotea crawls more slowly than Asellus and keeps more closely to the substratum, so that it is better able to adhere to a fairly smooth surface than Asellus. It was not often swept off its feet by a current of 435 cm. per minute, and it moved about in the current with greater ease than Asellus.

With Asellus the direction of the current could be readily determined at almost any time during the beginning of an experiment by examining the direction in which the animals within the trough were headed. This was not so with Cæcidotea, for often, so far as their positions were concerned, one would not suspect that there was a current. Cæcidotea, while remaining on the copper gauze partitions of the trough at times, did not do so as persistently as Asellus did.

After a time in most of the cases in which reactions did not appear promptly, Cæcidotea gradually collected toward the upper end of the trough. The maximum rheotactic response, *i.e.*, the collecting of the maximum number in the upper end of the trough, with one exception occurred within $\frac{3}{4}$ to 5 hours after the experiment began and averaged about 2.1 hours. This rheotactic

response persisted in most cases as long as the experiment lasted, and in one instance it lasted three days.

In some experiments the animals appeared altogether indifferent to the current and either moved about freely or tended to remain in the lower half of the trough. Two such results (experiments Nos. 2 and 6) may have been due to poor conditions of the animals, which had only 2 or 3 days before arrived from the caves of Indiana. Vigorous animals nearly always gave a definite and persistent response. A record of some of the experiments of this series is given in Table IX.

As compared with Asellus, Cæcidotea does not respond to the influence of a current so quickly; the maximum response with the former appears on an average in about 1.4 hours after the experiment starts, with the latter in about 2.1 hours. But the response of Cæcidotea continues longer than that of Asellus and usually lasts indefinitely.

In the experiments combining the effects of light and currents of water, where the upper end of the trough was darkened and the lower end subjected to strong illumination, both Asellus and Cæcidotea collected pretty generally in the upper darkened end. With Asellus this response reached its maximum in about three-quarters of an hour; with Cæcidotea in about an hour. After about an hour Asellus seemed to remain less generally in the dark end than before, while with Cæcidotea the reaction usually persisted without much diminution as long as the experiment was continued.

When the lower end of the trough was darkened and the upper strongly illuminated both Asellus and Cæcidotea showed tendencies to enter the upper end in spite of the light. With Asellus this tendency was very pronounced for a time, but the animals became so active on entering the light area that they were often carried off their feet and swept back into the lower end again. If Asellus was not swept back into the lower end it crawled back after a time and the rheotactic stimulus finally failed to bring it again into the upper end. Cæcidotea for a time entered and moved about in the upper light end to some extent. This persisted for various lenghts of time so that on an average several

TABLE VIII

In this table are given some details of the experiments on Asellus with currents of water. The number of the experiments, the date, the number of animals used, the strength of the current in centimeters per minute and the number of individuals in the upper end of the trough at the beginning of the experiments are given in the first five lines. In the succeeding lines are given the numbers in the upper end of of the trough at the various periods during the experiment.

NUMBER OF EXPERIMENT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
DATE	NOVEMBER 8, 1906	NOVEMBER 12, 1906	NOVEMBER 15, 1906	NOVEMBER 16, 1906, I	NOVEMBER 19, 1906, II	NOVEMBER 16, 1906, III	NOVEMBER 20, 1906, I	NOVEMBER 20, 1906, II	NOVEMBER 21, 1906, I	NOVEMBER 21, 1906, II	NOVEMBER 22, 1906	NOVEMBER 23, 1906	EXPERIMENT CONTINUED FROM PRECEDING DAY	NOVEMBER 24, 1906	NOVEMBER 26, 1906	NOVEMBER 27, 1906	NOVEMBER 29, 1906	SECOND DAY, EXPERIMENT CONTINUED FROM PRECEDING DAY	THIRD DAY, EXPERIMENT CONTINUED FROM PRECEDING DAY	DECEMBER 4, 1906, I	DECEMBER 4, 1906, II	ANIMALS ALL DRIVEN TO LOWER END AND OBSERVATION CONTINUED	EXPERIMENT CONTINUED FROM PRECEDING DAY
NUMBER OF ASELLUS USED	10	10	10	10	10	10	10	10	10	10	10	10		10	10	10	10			10	10		
STRENGTH OF CURRENT IN CM. PER MIN.	435	145	140	140	140	140	140	140	140	140	140	140		140	140	435	435			435	140		
Number in upper end at start	2	5	5	5	4	5	5	5	5	5	5	5	6	5	5	5	5	8	8	5	5	0	0
After 5 min	4	8	–	8	7	7	9	8	7	7	2	3	–	3	3	2	–	–	–	5	5	3	–
10 min	4	8	–	7	7	4	6	9	6	6	6	2	–	4	–	2	–	–	–	6	3	3	–
15 min	5	9	–	5	9	7	8	–	9	4	2	2	6	8	7	3	–	9	–	6	6	5	0
½ hr	4	–	3	0	7	4	8	6	6	–	5	5	4	9	9	7	–	–	8	7	8	5	–
¾ hr	6	8	–			6	7	3	4	–	8	6	5	5	7	8	–	9	–	6	7	2	–
1 hr	7	–	–			–	7	4	6	–	9	5	5	7	9	8	–	–	8	5	5	7	–

1¼ hr	7	—	—			—	3	—	—	—	7	8	5	8	2	7	8	—	—	6	8	—	—		
1½ hr	8	5	—			—	2	—	—	—	—	7		5	2	6	8	8	—	7	8				
1¾ hr	7	6	1			—	5	—	—	—	6	7		6	6	6	—	—	8	7	5	—	—		
2 hr	9	7	2			—		—	—	—	—	8		9	4	4	—	—	—	6					
2¼ hr	10	9	[5] 2			—		—	—	—	5	9		5	4	—	6	—	—	6	[6]	10	—		
2½ hr	—	7	2			3		5	2	—		7		—	3	—	—	6	7	4		7	—		
2¾ hr	—	7	2			—		4	—	6		5		—	—	—	—	—	—	5		7	—		
3 hr	—	8	3			—		—	3			5		8	—	—	7	—	7	—		—	—		
3½ hr	7	7	3			—		—	—			—		7	—	—	9	7	7	—		5	—		
4 hr		—	7			—		—	—			8		8	—	3	[5]	—	—	6		4	—		
4½ hr		—	5			8		—	—			—		9	3			—	—	4		4	—		
5 hr		7	5			—		2	—			6		9	3			—	7	3		—	—		
5½ hr			—			8			2			—		8				9	4	3		3	—		
6 hr			4						2			10		6				—	—	—		5	—		
6½ hr												—		—				—	6	6			—		
7 hr												9		8				10	—	—			2		
7½ hr												—						—	5	5					
8 hr												7						9	6	—					
8½ hr																		[5]	2	7					
9 hr																			2						
The following morning	1	9				8				1	2	6				0	8	7	2			0			
Time in hours after start until max. response occurred	2¼	¼	?	?	¼	4½	¾	⅛	¼	1½	1	2½		4½	½	¾	1¼			1½	1¾	2¼			
Time in hours after start until response decreased	3½	?				24+	¾	½	¾	¼	1¼	8		6	1¼	1¼	48			4½	4¾	3½			

Average time until maximum response 1.4 hr.

Average time until response decreased 7 hr.

[5] Experiment was continued over night and the following day, the record of which is given in the adjoining column.

[6] The animals were driven to the lower end of the tank and further observations recorded in the adjoining column.

TABLE IX

This table gives the details of the experiments on Cœcidotea with currents of water. The plan of this table is like that of Table VIII already described.

NUMBER OF EXPERIMENT	1	2	3	4	5	6	7	8			9		
DATE	NOVEMBER 6, 1906	NOVEMBER 9, 1906	NOVEMBER 13, 1906	NOVEMBER 19, 1906	NOVEMBER 26, 1906	NOVEMBER 28, 1906	DECEMBER 18, 1906	DECEMBER 19, 1906	SECOND DAY, EXPERIMENT CONTINUED FROM PRECEDING DAY	THIRD DAY, EXPERIMENT CONTINUED FROM PRECEDING DAY	DECEMBER 20, 1906	EXPERIMENT CONTINUED FROM PRECEDING DAY	EXPERIMENT CONTINUED FROM PRECEDING DAY
NUMBER OF ANIMALS USED	10	10	10	10	10	10	10	10			10		
STRENGTH OF CURRENT IN CM. PER MIN	435	435	140	134	140	435	435	435			435		
Number in upper end at start	7	1	5	6	6	6	7	2	6	4	5	6	6
After 5 min	7	1	4	6	7	7	6	—	—	—	—	—	—
10 min	7	1	5	—	5	7	6	—	—	—	—	—	—
15 min	7	1	5	—	5	7	6	—	—	4	—	—	—
½ hr	6	1	6	—	3	—	7	—	6	—	—	6	6
¾ hr	8	2	6	—	2	—		—	—	—	—	—	—
1 hr	7	1	4	8	3	6		—	—	—	—	—	—
1¼ hr	6	3	7	9	4	—		7	—	—	—	—	—
1½ hr	5	2	—	9		—		—	—	4	—	—	—

After 1¾ hr.	6	1	—	9		6		—	—	—	—	—	—	
2 hr.	6	3	—	9		4		—	—	4	—	—	—	
2¼ hr.	5	4	9	9		—		7	—	—	—	—	6	
2½ hr.	—	2	6	9		5		—	7	—	—	—	—	
2¾ hr.	—	2	8	9		5		—	—	—	—	—	—	
3 hr.	—	—	8			5		9	—	3	6	6	—	
3½ hr.	6	1	7			—		—	—	—		—	—	
4 hr.		1	8			1		8	6	—		—	7	
4½ hr.		—	8			2		—	—	4		7	—	
5 hr.		—	10			—		—	—	—		—	—	
5½ hr.		—	9			7		7	—	—		—	—	
6 hr.		1	—			5		—	6	—		—	—	
6½ hr.		—	9			—		—		—		—	—	
7 hr.		—	8			—		—		—		6	—	
7½ hr.		—				—		—		—		—	—	
8 hr.		—				5		—		—		8	10	
8½ hr.		1				—		—		—				
9 hr.		—				5		7		4				
The following morning	8		10	6	7	5		6*	4*		6*	6*		Averages.
Time in hours after start until max. response occurred	¾	?	5	1¼	?	?	½(?)	3			32			Without exp. 9,—2.1. 7.1
Time in hours after start until response decreased	no decrease in 24		no decrease in 24	?				5½			no decrease in 56			27.4+

* 8 Experiment was continued over night and during the following day, the record of which is given in the adjoining column.

remained in the upper end for about three-quarters of an hour, after which time the animals that entered the light seldom remained long. Cæcidotea was not often swept back. It wandered about in the light upper end for a time and entered the dark end again apparently by accident. Often, too, it turned back into the dark as soon as it came to the light area.

Under the last conditions of experimentation Asellus seems more rheotactic at first than Cæcidotea, but this reaction persists for a shorter time than in Cæcidotea. Probably a better way of describing this state in Asellus is that at first its rheotaxis is stronger, as compared with its negative phototaxis, than Cæcidotea's, but that its rheotaxis soon gives place to negative phototaxis; while Cæcidotea does not so soon cease to enter the light as Asellus, it never enters and collects in the light in large numbers. Its negative phototaxis is from the first stronger than its rheotaxis.

In regions of caves the cave streams, as I know them, ordinarily originate underground and flow out of the caves, although there are many exceptions to this rule. In Mayfield's Cave, near Bloomington, Indiana, which is the source of some of my material, there is no inflow into the cave from above ground except through sink holes in times of heavy rains or thaws. The stream is merely an outlet for underground drainage.

The experiments in which the upper end of the trough was darkened while the lower remained light, thus resembled in miniature the conditions met with in this cave. Judging from the results of these experiments Asellus and Cæcidotae ought both to enter such a cave, but Asellus being less persistently rheotactic would be less inclined to follow up the stream and remain within the cave. Cæcidotae, while less strongly rheotactic at the beginning, is persistently so, and hence would tend to creep persistently into the cave. The added fact that Asellus, after having been in darkness for some hours, is positively phototactic would induce the animals to leave a cave after once having become indifferent to the current, provided that they were carried by accident to within a glimmer of light.

The experiments in which the lower end of the trough was

darkened and the upper end illuminated afforded conditions somewhat resembling conditions in caves which are entered by streams from the outside. Under such conditions, judging from the results of the experiments, Asellus, when carried to the inlet, would tend to leave the cave because of its relatively stronger rheotaxis. In addition to this factor, however, the positive phototaxis of Asellus after having been in darkness would also aid. Cæcidotea, on the contrary, is always negatively phototactic, and while it shows a tendency under these circumstances to enter the light because of its rheotaxis, it is probable that this form of reactions is always subordinate to its negative phototaxis.

III. STUDY OF THE FOOD

Attempts were made to ascertain if a difference in the food of Asellus and Cæcidotea was possibly an important factor in determining the habitats of the two species. To this end specimens of Cæcidotea were taken direct from the caves in Indiana and preserved in formaline before they had an opportunity to feed upon other substances than those they might get in their normal surroundings and the same was done with Asellus communis obtained near Cambridge, Mass.[10]

The digestive tracts of each were then carefully examined to determine their contents.

Afterward several individuals of each species were kept in clean tap water without food for several days and then placed in separate jars with bits of decayed leaves and living Ceratophylum and subsequently examined to determine what was taken for food when living and dead plant tissue were equally present.

With Cæcidotea the evidence pointed to the conclusion that the animal in the cave collects for food whatever decaying organic matter it happens upon. Naturally dead plant tissue forms

[10] Mancasellus tenax dilata, the common and extremely abundant Asellid from southern Indiana, was similiarly treated and examined. It was found that the food of this species from the region about the cave in Indiana is practically the same as that of Asellus communis near Cambridge, Mass.

the main part of its food. The digestive tracts contained relatively small amounts of mineral matter. Those placed with living and decaying plant tissue ate relatively small amounts of green algæ, probably only that taken in with the decayed plant tissue.

From the evidence obtained from the series of examinations of Asellus it seemed probable that it takes its food either from surfaces of dead plant tissue or from living plants. In cases where material apparently quite recently taken as food was found in the digestive tract there was always more or less fresh plant tissue and those individuals which were deprived of food for a time and then placed with both the decaying leaves and the living Ceratophyllum seemed to have collected nearly all their food from the Ceratophyllum. There was, however, no evidence to prove that green plant tissue was a necessary portion of the food of Asellus. It probably could live upon decaying organic matter exclusively, as Cæcidotae does, but it partakes of both kinds of food and will use living plant tissue largely when both living and dead are present.

In every case the digestive tracts of those Asellus examined contained a large percentage of mineral matter, in most cases as much as 85 per cent of the contents being inorganic. It will be remembered that the digestive tracts of even those Cæcidotea which were taken directly from the caves contained very little mineral matter.

The difference between the amounts of mineral matter taken with the food in the two species is remarkable in caves where Cæcidotea obtains its food, organic matter is extremely scarce, while, where Asellus lives, the proportion of organic to mineral matter is very many times as great. This difference suggests that Cæcidotea possesses a superior discriminative power in selecting its food. Such ability would be of immense advantage to an animal in a cave. The lack of discrimination in selecting food clearly indicates, I believe, that Asellus is incapable of meeting the food conditions within caves.

IV. GENERAL DISCUSSION

The significance of the various separate results obtained in the different series of experiments has in several cases been pointed out in summarizing these results.

Cæcidotea stygia and Asellus communis have quite similar habits, except for the fact that the former lives almost exclusively in subterranean waters, whereas the latter, though occasionally found in cave water, is extremely rare there. Its occurrence in caves has been mentioned only a few times. When collecting in caves I have never seen either Asellus communis or Mancasellus tenax dilata, although both species occurred near caves in which I did much collecting, and the latter species was present in immense numbers immediately outside the caves. Cæcidotea, when found above ground, has occurred only in water near and immediately connected with underground waters.

These two animals, though differing in habitat, are much alike in many of their reactions to stimuli. Both are negatively phototactic to such intensities of light as they respond to at all; both respond in like manner to various tactile and mechanical stimuli, and the regions of the body most highly sensitive to these stimuli, are nearly the same in the two species; both are rheotactic, responding to currents of water in like manner. When subjected to the influence of a current of water, in a trough one end of which was strongly illuminated, both species, though for a time responding to the rheotactic stimulus alone, soon, in most cases, reacted to the light alone. Their food is nearly the same, both species feeding largely upon decaying plant tissue.

The following differences in the reactions of the two species were noted:

1. Asellus is decidedly more responsive to light stimuli and responds to much lower light intensities (2.5 C. M.) than Cæcidotea (80 C. M.); after retention in darkness Asellus is for a time positive to a considerable range of intensities (2.5 C. M. to 80 or more C. M.). Cæcidotea is never positive.

2. Cæcidotea, on the other hand, is decidedly the more respon-

sive to mechanical stimulation, this difference appearing in the number and vigor of its responses and in its having a decidedly lower threshold of stimulation than Asellus.

3. Cæcidotea, though at first less rhetotactic than Asellus, is persistently rheotactic, whereas rheotaxis with Asellus is only temporary. When the stimulus of strong illumination in the lower part of the trough was added to the stimulus of a current, the Cæcidotea more persistently remained within the upper dark end.

Although there are considerable differences between the responses of the two species in minor details, these are, after all, not so much differences of kind as of degree. Physiologically considered, the two species are very much alike.

The relatively slight sensitiveness to light shown by Cæcidotea, as compared with Asellus, is what one might expect from the responses of eyeless animals in general, as compared with those which possess eyes, For example, Dubois ('89, pp. 358–359) found that Proteus, the blind salamander of the European caves, responded to light from a projection lantern. Semper ('89, p. 79) states that Proteus is sensitive even to daylight. Eigenmann ('00, pp. 113–116) found the blind fish, Amblyopsis, sensitive to strong light suddenly thrown upon it as well as to diffuse daylight, for these animals, when kept in a pool in the open air, where they remained concealed among rocks during the day, swam about freely in twilight and at night. It has been shown by Payne, ('07) that Amblyopsis reacts to light from a "100 c.p." acetylene lamp at 32 inches from the end of the aquarium; but with this intensity of illumination an average of only 54 per cent of the individuals were in the end farther from as against 46 per cent in the end nearer to the light. With considerably greater intensities he found the reactions were much more decided. Hence it seems that the lower intensity used by Payne ('07, p. 318) is near the threshold of stimulation for Amblyopsis.

There is no reason to suppose from any of these observations that such animals respond to low intensities of illumination.

The nicety with which even slight sensitiveness to light may regulate an animal's movements is well illustrated by the following case. Parker ('05, p. 418) found sensitiveness to strong light in the skin of the tail of Ammocœtes, and pointed out the significance of this in relation to the burrowing habit of the animal. Here again, the sensitiveness was to intense light only. The eyes of these animals probably subserve all light-receptive functions necessary for swimming about, but in burrowing the integumentary organs of the tail serve to distinguish intensities sufficiently to direct the animal to continue burrowing until completely covered. Here, as in the other cases cited, the organs are not highly discriminating light-receptive organs, but they suffice for the regulation of the animal's movements.

Among the higher animals those that possess degenerate, or poorly developed eyes are such as in general live in dark situations. Their light-perceiving organs are of advantage to them only in aiding them to remain within a suitable environment. Highly developed light-receptive organs are of no advantage in their dark habitat, and organs capable of perceiving only considerable changes in intensity of illumination are sufficient to serve as a check to keep them in their proper surroundings.

Cæcidotea nearly always lives in absolute darkness and ordinarily has little occasion to discriminate between intensities of illumination. If, however, in approaching the mouth of a cave it becomes subjected to increased illumination, its light-receptive organs ordinarily are discriminative enough to prevent its leaving its subterranean abode. Whether its light-perceptive organs were adapted to its cave habitat or whether its cave habitat was adopted because its organs suited it for such surroundings, does not here concern us. At any rate, Cæcidotea's light-receptive organs are sufficiently discriminating to serve their part in regulating the movements of the organisms within caves.

We have no reason to suppose that a species lives where there is extremely little light because it is extremely responsive to light. On the contrary, we have every reason to suppose the opposite—that when an animal lives where there is little light and where it has little opportunity or occasion to be influenced by light, it will

be little responsive to this influence. It is a part of the adaptive economy of nature, a frugality long noted with reference to eye structures, but apparently less remarked with regard to light perception in general.

Such animals as the cockroach and Oniscus (cf. Cole, '07, pp. 373–380) remain in relatively closely circumscribed and restricted dark situations by day. While their habits are such that highly discriminative eyes would be of little use to them, slightly acute organs for discriminating differences in intensity serve them very well, aiding them in reaching and remaining concealed within their diurnal haunts. Asellus often lives in fairly exposed situations, but, like Oniscus, it tends to seek the darker of the available regions. It possesses better powers of discrimination than Cæcidotea, and this serves it well in aiding it to find quite small and restricted shaded areas, which Cæcidotea can not do.

The experiments discussed in this paper clearly show that the subterranean species, Cæcidotea stygia, has greater sensitiveness to mechanical stimulation (whether purely tactile or vibrations) than its near relative, Asellus communis, which lives above ground. Cæcidotea proved decidedly the more sensitive, both in having a lower threshold of stimulation and in responding more generally and more vigorously to such stimuli. Hence these isopods furnish an undoubted case in support of the common belief that cave animals have acquired greater sensitiveness to mechanical stimulation than their near relatives living in other situations.

Since Cæcidotea was shown to be much less sensitive to light than Asellus, its greater sensitiveness to mechanical stimulation is a good illustration of the principle of increase in sensitiveness to one sort of stimulation in compensation for the partial loss of sensitiveness to another, *i.e.*, the organism is so adapted to its environment that when one influence ceases to regulate its movements another acquires increased importance and in a measure replaces it.

There still remains one point to be considered—the bearing of these experiments on a possible explanation of the occurrence of the cave species within caves rather than outside of them and the

occurrence of non-caverniculous species outside of rather than within caves.

One needs to experiment with the two species for only a short time to be struck with the similarity of their responses to various influences. The minor differences in their reactions, however, are very significant in relation to the habitats of the animals. While Cæcidotea is responsive to only fairly high intensities of light (80 C.M. or greater), it is always negative to any intensity to which it responds at all. Hence, if outside a cave, its light reactions alone would tend to lead it into a cave if there were one near, while if it were in a cave and wandered into the light near an outlet, its negativity to light would prevent its leaving the cave and passing into waters above ground. If Asellus were near a cave, its response to light would at first tend to direct it into the cave. But, after having been in darkness within the cave for a time, it would again become positively phototactic, so that if it came in reach of light from the outside it would escape.

Again, Cæcidotea, while less strongly rheotactic than Asellus, when first subjected to a current, is more persistently rheotactic. In experiments combining the effects of a current of water with light stimulation under such conditions that when the animals moved against the current they passed into a darkened region, it was shown that Cæcidotea remained in the darkened region more persistently than Asellus did. This, as already stated, repeats in miniature the usual conditions found in cave streams, since they generally flow out of caves rather than into them. In such cases, even if Cæcidotea were not rheotactic, its greater tenacity in holding to the substratum would better enable it to make its way into caves and remain there; whereas, if the stream were swift, Asellus could not hold its own against the current. In the experiments with light combined with a current of water under such conditions that when the animals moving against the current passed out of a darkened region into a strongly illuminated one, the other cave stream condition (found in streams which enter caves from above ground) was in a way duplicated. In those experiments Asellus was more vigorously rheotactic at first, but Cæcidotea more persistently so, although under these condi-

tions neither persisted in entering the light for any great length of time. Asellus, however, after having been in a cave in darkness for a time, would become positively phototactic and would then have both its rheotactic and its positively phototactic impulses to direct it out of the cave.

The suggestion that cave conditions involve fewer mechanical disturbances in underground waters than are found in out-door streams and pools, and that the relative immunity from mechanical disturbance within caves is a factor in determining the distribution of the one species within caves and the other outside, seems hardly worth following up.

The difference in the food taken by the two species seems to be a factor in determining their habitats. Cæcidotea eats nearly exclusively dead plant tissue, even when provided with living and dead tissue in equal abundance. While Asellus takes much fresh plant tissue in addition to the dead plant tissue which it also feeds upon, it is not shown that living plant tissue forms a neccessary part of its food, so that apparently it might feed upon dead plant tissue exclusively. There is an extremely small amount of organic matter and a great prevalence of sand, etc., in caves as compared with the amounts of organic matter and inorganic matter in the situations in which Asellus lives. In view of this fact the relatively very small amount of dirt and small particles of mineral matter within the digestive tract of Cæcidotea as compared with that of Asellus suggests that Cæcidotea exercises more discrimination than Asellus in taking its food.

The importance to an animal living in a cave of a superior ability in selecting its food could not be overestimated, since the organic matter there is so scanty. If Cæcidotea possesses such an advantage over Asellus that alone may be a very important factor in determining the suitability of Cæcidotea to a cave habitat and the unsuitability of Asellus for the same locality.

To recapitulate, we find some apparent importance in the factors of the animals' food and their different rheotactic responses in determining the habitats of the two species, but the one factor which seems of most importance, and which alone affords an explanation of Cæcidotea's living in caves and Asellus not living

in caves, is the different responses to light,—Cæcidotea always responding negatively to such intensities as it responds to at all, and Asellus, after having been in darkness, being positively phototactic for a time.

Since Asellus is so closely related to Cæcidotea morphologically and physiologically, it would seem that if under stress of circumstances any epigeal animal could suddenly become a cave inhabitant, Asellus might be expected to be capable of undergiong such change in environment. Asellus is in many ways apparently nearly suited for cave habitation, and in time it may become further modified so that it will be capable of living in caves, but it is not fitted for cave life. Its physiological reactions are such that it is prevented from taking up cave life, and further its apparent lack of discriminative ability in selecting food renders most improbable the continued existence of a straggler of this species within a cave. This case fails to support Lankester's ('93, p. 389) theory of the sudden and accidental origin of cave animals, but rather lends support to the theory that cave animals must become closely adapted for cave life before they are capable of taking up such existence.

V. SUMMARY

Mechanical Stimulation

1. Asellus and Cæcidotea respond in much the same manner, and corresponding parts in the two species are the regions of greatest sensitiveness.

2. Cæcidotea is decidedly more responsive than Asellus, reacting more often, more vigorously, and to weaker stimuli.

3. The threshold for Cæcidotea is below that for Asellus.

Rheotaxis

1. Asellus and Cæcidotea are rheotactic and respond to currents in like manner.

2. Cæcidotea, though at first less rheotactic than Asellus, is persistently rheotactic, whereas the rheotactic response with Asellus is only temporary.

Photic and Rheotactic Stimulation

1. When subjected to the influence of a current of water in a trough the upper end of which is strongly illuminated, both species, though for a time apparently responding to the rheotactic stimulus alone, soon react principally to the light.

2. When the upper end of the trough is darkened, Cæcidotea more persistently remains within the darkened upper end.

Food

1. Cæcidotea and Asellus take about the same food, but Asellus eats much live plant tissue with the decaying plant tissue.

2. Asellus takes in vastly more debris and particles of mineral matter with its food than Cæcidotea.

General Habits

1. Cæcidotea stygia and Asellus communis are not only structurally similar, but their habits and reactions to various stimuli are very much alike although there are several minor differences in their reactions.

2. The cave species is decidedly less sensitive to light than its above-ground relative.

3. Cave animals do not have need for highly discriminating light-receptive organs. Their movements are well regulated by light-receptive organs which are capable of distinguishing only considerable intensities of illumination, so that when approaching the outlet of subterranean waters their negative response to light restrains them from passing beyond the limits of caves.

4. The subterranean Cæcidotea is clearly very much more sensitive to tactile stimulation than its epigeal relative.

5. Cæcidotea, being less sensitive to light stimulation than Asellus and more sensitive to mechanical stimulation, affords an illustration of compensative sensitiveness to one influence for a partial loss in sensitiveness to another influence.

Habitat

There are several possible factors which determine the habitat of one of these species to be within and the other outside of caves.

1. There is a remote possibility that the relative freedom from mechanical disturbance within cave waters is a factor in determining the existence of Cæcidotea in caves.

2. The difference in the reactions of Asellus and Cæcidotea to light affords an explanation of the occurrence of Cæcidotea in caves, and subterranean waters in general, and the virtual non-occurrence of Asellus in such situations; for the negative response of Cæcidotea to light would aid in directing it into caves and keeping it there; but Asellus after being in darkness becomes positive, and therefore would move toward the light, *i.e.*, out of a cave in case it had by chance made its way into one.

3. Cæcidotea, being more persistently rheotactic than Asellus, and being able in creeping to hold its own against a current better than Asellus, would not readily be swept away by a stream flowing out of a cave. Asellus, on entering a cave under such conditions, would soon lose its rheotactic response and further would become positively phototactic in a few hours. Hence, if it by chance came near the mouth, it would react to the light and escape.

4. The reactions to the influence of a current of water combined with the influence of light afford an additional factor in determining the distribution of the two animals.

5. An apparently superior discrimination on the part of Cæcidotea in selecting its food may make it possible for Cæcidotea, rather than Asellus to live in caves where organic matter is extremely scanty.

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EFFECT OF CHEMICALS ON GROWTH IN PARAMECIUM

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With One Figure

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INTRODUCTION

Most studies of growth have been made upon organisms composed of a multitude of cells, so that the growth observed is a complex resultant of the growth of the component cells. It is evident that in producing such a resultant, the method of growth of each single cell might be very different from that of the organism as a whole. The present paper examines the growth of a single cell, as affected by various agents, particularly chemicals.

There have been but few studies of the growth of single cells. Jennings[1] ('08) studied the normal growth of the infusorian Paramecium, showing that it follows a curve that is similar, in a general way, to that of the growth of a multicellular animal having determinate growth. Popoff ('09) investigated the relative growth of nucleus and cytoplasm in a number of infusoria. Balls ('08)) examined the growth of the Sore-shin fungus, particularly the effect of temperature upon it. In this organism the growth of the cell is indeterminate, as in many of the higher organisms.

The common infusorian Paramecium has served as object of the present study. The normal growth of Paramecium is thus summarized by Jennings:[2]

In the following investigation, I have endeavored to find out some of the factors determining growth in Paramecium, and to discover whether size may be permanently modified by external conditions. The effect of the following chemicals upon the growth of Paramecium has been studied: sodium chloride, the alkaloids nicotine and strychnine nitrate, and alcohol. The growth in pure water, containing no food or salts, has likewise been studied.

I wish here to acknowledge my indebtedness to Prof. H. S. Jennings, under whose direction the work was done, for valuable suggestions and criticism. I also wish to thank Prof. B. E. Livingston for his interest in the experiments on osmotic pressure.

[1] Loc. cit., page 441.

[2] Loc. cit., page 448.

MATERIAL AND METHODS

In all the experiments one pure line of Paramecium has been used, that designated by Jennings[3] as I_2. This was the largest race he isolated, measuring from 200 to 230microns, orabout one-fifth of a millimeter. All the specimens used are descended from a single pair of conjugants which he selected from a wild culture. Thus, by using animals all derived from a single pair, any possible differences due to diverse ancestry are excluded and only variation within a pure line has to be dealt with. The Paramecia were kept in stock in large flat dishes holding about 2 to 3 liters. The hay infusion in which they were kept was made fresh about once a week, by pouring off some old infusion and adding new hay and fresh water. The Paramecia were thus kept in good condition and afforded an abundance of material at all times.

Paramecia undergoing division were picked out from the stock culture with the capillary pipette, and put on a slide with a concave depression. At the moment when the two halves separated, one of the daughter cells was placed in a few drops of hay infusion in one concavity on a ground glass slide, the other daughter cell was placed in a similar concavity on the same slide, this latter depression containing the solution whose effect was to be studied. The time at which the halves were isolated was noted, and after a certain interval they were taken out, killed and measured separately. This gives a direct method of measuring growth, as the specimens, can be killed at any age desired. The control in hay infusion gives a normal curve of growth and acts as a check, while the other shows directly the effect of the solution upon the normal growth. The fact that the two halves come from the same mother cell gives an almost ideal condition of experimentation, since the two organisms studied are of the same age, their previous history is the same, and they are in the same physiological condition at the moment of separation.

The Paramecia were taken out by means of the capillary pipette, gathered into one or two drops of liquid and then killed by sud-

[3] Loc. cit., page 494.

denly adding a large amount of Worcester's solution (a saturated solution of $HgCl_2$ in 10 per cent formalin). When Paramecia are properly killed by this method no distortion takes place. They were then measured by projecting them with the Edinger projection apparatus, and measuring the image with a millimeter scale. Lenses of such combinations were used that three microns in the object were equal to one millimeter in the projected image. The measurements were then converted into microns by multiplying by three.

GROWTH IN DISTILLED WATER

Before studying the effects of chemicals as upon growth it is well to examine growth in a fluid containing as small an amount of dissolved chemicals as possible; that is, distilled water. Any growth taking place in distilled water will evidently not be due to food materials present in the medium nor is it likely to be due to any specific salts, since these are almost lacking. After the nature of growth in distilled water has been determined, the specific effects of substances dissolved in the water can be studied.

Daniel ('08) showed that if Paramecia are introduced directly from hay infusion (which has a high salt content) into pure distilled water, it is the sudden change that injures them. He also showed that they could be made to live in pure distilled water if they were introduced into it gradually.

The distilled water used in the experiments was made by Dr. G. F. White of the Johns Hopkins Chemical Laboratory. Ordinary laboratory distilled water was redistilled from sulphuric acid and potassium chromate, then distilled from barium hydrate and condensed in block tin. This water gives a conductivity of about 2×10^{-6}.

Effect of Pure Distilled Water

In the experiments, one half of a dividing specimen was placed in hay infusion, such as was known to be favorable to growth: the other was placed in pure distilled water. Before transferring

to the distilled water, the specimen was washed once in a considerable quantity of the same distilled water, in order to remove any trace of hay infusion remaining on it. At the end of five minutes specimens in the hay infusion had increased in length from 139.5 to 153.3 microns. Those in the distilled water had grown precisely the same amount. Now, however, the injurious action of the distilled water began to show by the fact that the specimens in it began to shorten and thicken, while those in the hay infusion continued to increase in length and decrease in thickness. At the end of fifteen minutes the control specimens measured 159.6 × 42.7 microns, those in the distilled water 149.7 × 53.1 microns. The latter are thus actually shorter than they were ten minutes earlier. At the end of thirty minutes they were all dead, while those in the control were strong and active. The comparative measurements are given in Table I.

TABLE I

Comparative measurement in microns of Paramecium in pure distilled water and in hay infusion

AGE	CONTROL HAY INFUSION			PURE DISTILLED WATER		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.5	42.6	9			
5	153.3	44.0	12	153.3	47.0	12
15	159.6	42.7	13	149.7	53.1	13
30	Normal		15	All dead	Swollen	15

It is notable that a certain amount of growth did occur in the distilled water in the first five minutes, in spite of the fact that the latter is quickly injurious and soon fatal. It seems clear that the injurious result was here due, as in Daniel's experiments, to the sudden change from the fluid with the high salt content to one with a low salt content.

Effect of Adding NaCl to the Distilled Water

The effect of adding a small amount of some salt to the distilled water was next studied. For this purpose sodium chloride was used. When enough sodium chloride was added to the distilled water to give a $\frac{N}{100}$ solution, the results are those given in Table II.[4]

TABLE II

Comparative measurements in microns of Paramecia on $\frac{N}{100}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{100}$ NaCl		
	Length	Width	No. of Specimens	Length	width	No. of Specimens
Min.						
0	139.5	42.6	9			
30	167.2	38.7	8	164.3	39.3	9
90	177.3	37.9	14	169.5	37.5	14
Hours						
5	185.7	41.6	15	165.0	37.2	15
24	174.9	54.9	(10to)17	184.8(5) 5 alive	48.6(5) 5 dead	10

From this table it is evident that the addition of the small amount of the NaCl to the distilled water has a very good effect on the vitality and growth of the animals. Most of them are alive at the end of twenty-four hours. The growth is only slightly retarded in the salt solution as compared with that in the hay infusion. At the end of twenty-four hours, however, half the animals in the salt solution were dead or dying. The living specimens in the salt measured 18.48×48.6 microns, and were thus larger than the controls, which were 174.9×54.9 microns. But it must be noted that seven of the ten specimens that were in the

[4] In this and the following tables, whenever division has taken place, the figure enclosed in parentheses is the number of specimens at the beginning of the experiment, the other number indicates the number of specimens at the end of the experiment. In this case there were 10 specimens at the beginning of the experiment. These increased by division to 17 specimens at the end, all of which were measured.

control at the beginning had divided, giving seventeen specimens, and the difference in sizes is due to the fact that some of these seventeen specimens are young and not fully grown. I should say however, that the five in the salt were not quite so large as normal. Evidently growth that is almost normal in amount and rate can take place in a fluid that will not sustain life for any great length of time.

The effects in this experiment are explained by the one next performed. The animals were placed in pure distilled water plus a sufficient amount of sodium chloride to make a $\frac{N}{70}$ solution. The results are shown in Table III.

TABLE III

Comparative measurements in microns of Paramecia in $\frac{N}{70}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{70}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	148.5	50.7	8			
90	183.7	45.0	12	188.7	47.1	11
Hours						
5	200.1	58.2	14	177.3	45.9	12
24	199.2	63.9	(21 to) 27	211.5	60.9	21

As the table shows, growth in $\frac{N}{70}$ NaCl took place almost as well as in the hay infusion. At 90 minutes, those in the salt were as large as those in the control. At 5 hours, they had fallen somewhat behind, but at 24 hours they were of about the usual adult size. The lack of food had prevented them from dividing, while 6 of the 21 control specimens had divided, giving 27 altogether. The larger average size of the specimens in the salt solution was doubtless due to this division in the hay infusion; some of the specimens in the latter were young and not full grown.

Thus a $\frac{N}{70}$ solution of NaCl in distilled water has the necessary amount of salts for Paramecium to live in it with normal growth. The amount of salts in the $\frac{N}{100}$ solution was evidently not sufficient to keep the animals alive for a long time.

A $\frac{N}{50}$ NaCl solution in pure distilled water also gave interesting results, as shown in Table IV.

TABLE IV

Comparative measurements in microns of Paramecia in $\frac{N}{50}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{50}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.5	42.6	9			
30	171.6	37.8	10	164.1	40.5	10
90	182.0	37.8	15	166.6	37.5	15
Hours						
5	191.4	41.1	15	161.7	36.6	14
24	183.0	58.8	(10 to) 13	185.1	46.8	10

At the end of 30 minutes, the Paramecia in the $\frac{N}{50}$ NaCl solution were shorter than the controls. Growth was still retarded at 90 minutes and at 5 hours. At the end of 24 hours the specimens in the salt were long but not so thick as the controls. The controls, however, had increased from 10 to 13 specimens, so that those in the salt were only slightly retarded in growth. Those in the salt showed evidences of injury.

TABLE V

Comparative measurements in microns of Paramecia in $\frac{N}{20}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{20}$ NaCl.		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	146.4	46.2	10			
5	151.0	47.7	9	153.9	44.4	11
15	159.9	47.4	13	157.2	44.7	10
30	168.3	43.5	10	164.4	47.4	9
60	189.0	42.0	13	166.5 (2) 11 dead	43.5 (2) 2 alive	13

A $\frac{N}{20}$ NaCl solution in pure distilled water had no effect on growing Paramecia at 5 or 15 minutes of age (see Table V). At 30 minutes, those in the salt were slightly smaller than the controls; while at 60 minutes, of 13 specimens in the salt, 11 were dead and 2 were smaller than the controls.

To sum up the results with distilled water, we find that pure distilled water killed Paramecia that have been living in hay infusion by the great change from the high to the low salt content. When the Paramecia are put into distilled water with enough NaCl present to make it a $\frac{N}{100}$ solution, the injurious effect is not so marked and becomes very noticeable only at 5 to 24 hours. A $\frac{N}{70}$ NaCl solution in distilled water seems to have the necessary salt content to keep the Paramecia in normal condition. The $\frac{N}{50}$ and $\frac{N}{20}$ NaCl solutions in distilled water have a much higher salt content than is beneficial, and so cause a certain amount of inhibition of growth. More light upon this matter is given by the further work upon NaCl which is to be reported next.

GROWTH IN SODIUM CHLORIDE

Perhaps the chief purpose of the present paper was to study the effects on cellular growth of certain well-known poisons, particularly nicotine, alcohol and strychnine. In order that there should be no danger of mistaking for specific effects of these poisons symptoms that are likewise produced by other and unrelated chemicals, I first made a careful study of the effects of ordinary sodium chloride when added to the infusion in which the animals live. This substance, though not commonly accounted a poison, has of course most deleterious effects when present in too great amounts. These effects, particularly on growth, we shall now examine.

A $\frac{N}{10}$ NaCl solution was made by dissolving 0.146 grams of soldium choride in 25 cc. of hay infusion. This being the most concentrated solution of the salt that was used, any strength desired was made by dilution of this with hay infusion. The same hay infusion without the sodium chloride was used for the control experiments.

Preliminary Experiments on the Effect of NaCl on the Form and Dimensions of Adults

Adult Paramecia put into a NaCl solution died after some time. They first became thin, then shorter and much thinner, and slowly died. The appearance of Paramecia as they are dying is very characteristic; they become much swollen, sometimes twice as thick as normal, sometimes longer, or they may be shorter; the protoplasm contains many vacuoles; the animals swim about slowly. The exact moment of death is difficult to determine in this condition, as vitality seems to remain a long while, and even after they have become motionless, they may sometimes be made to move again by stimulating them with a capillary rod.

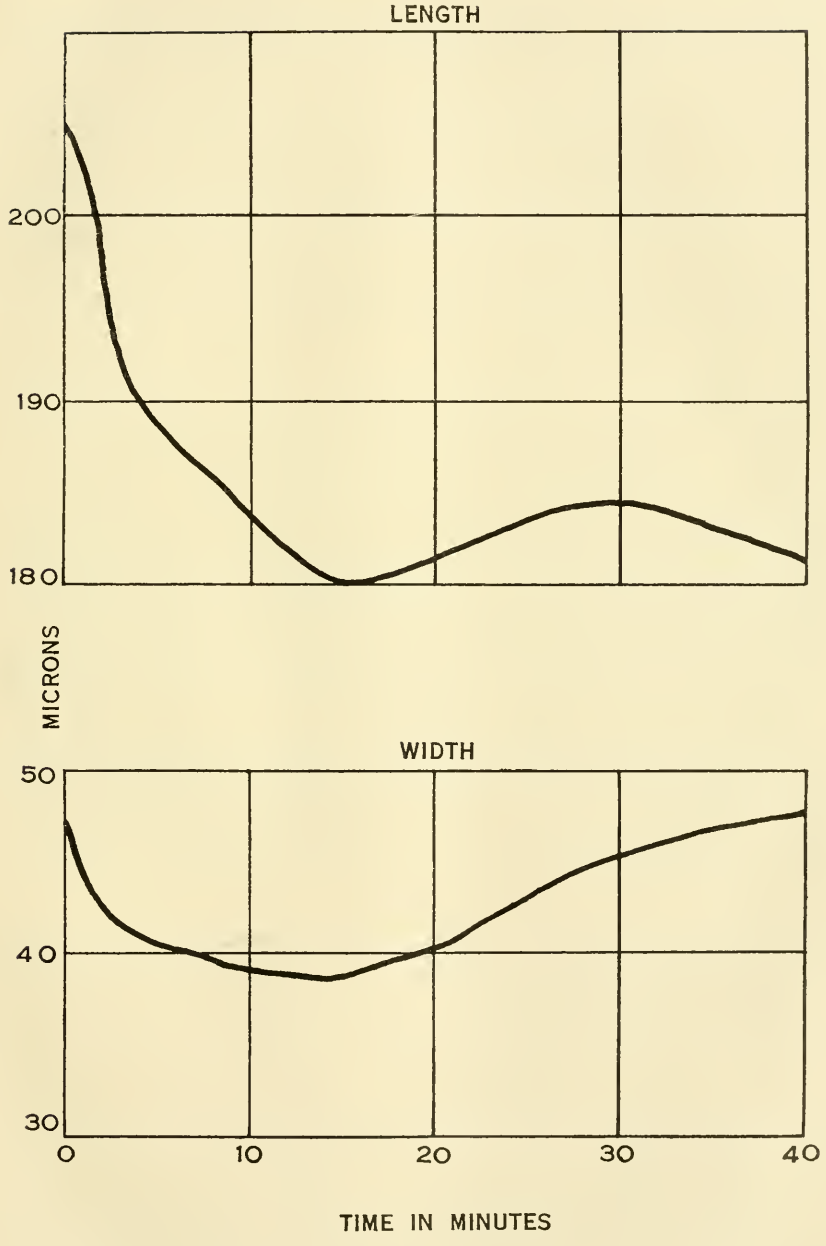
To determine the effect on dimensions, a large number of specimens were put into a $\frac{N}{10}$ NaCl solution (see table VI).

TABLE VI

Dimensions of adult Paramecia in $\frac{N}{10}$ *NaCl.*

TIME	LENGTH	BREADTH	NO. OF SPECIMENS MEASURED
Min.			
Normal at beginning	204.5	47.0	50
3	191.2	41.2	50
5	188.6	40.8	50
8	186.0	39.7	50
10	183.8	39.3	50
15	180.1	38.7	50
25	183.8	43.4	50
40	181.2	45.7	50

At intervals of 3 minutes, about 50 were taken out, killed and measured. Most of the animals died about 10 to 20 minutes after being put into the salt, but some, more resistant than the others, remained alive 60 minutes. The curve (Fig. 1), plotted from these figures, is thus based on different numbers of individuals


LENGTH
200
190
180
MICRONS
WIDTH
50
40
30
0
10
20
30
40
TIME IN MINUTES

in different parts; it is nevertheless instructive. The changes in the form and size of Paramecium shown by the curve, are the same as seen under the microscope. The first effect is an immediate decrease in the width, and at the same time, a shortening of the organism. Further decrease in length takes place, and then, as death approaches, the Paramecia become thicker and slightly longer. I have designated this form of curve as the death curve, as later work shows that its appearance is always followed by death.

Adult Paramecia put into a $\frac{N}{15}$ NaCl solution died in one-half to 3 hours, none living in this strength longer than 3 hours. The same general effects were observed as in the $\frac{N}{10}$ NaCl solution, except that, extending over a longer period, they were not so marked.

Adult Paramecia put into a $\frac{N}{20}$ NaCl solution became thinner for about 20 minutes, then gradually regained their normal size and continued to live normally in that solution.

In order to see if $\frac{N}{20}$ NaCl and the more dilute solutions of NaCl had any effect upon size in adult Paramecia which were kept in them for longer periods of time, the following experiment was tried: One hundred Paramecia taken from the stock culture were put into each of the following solutions, $\frac{N}{20}$, $\frac{N}{30}$, $\frac{N}{40}$ and $\frac{N}{50}$ NaCl, and a control composed of hay infusion (the same as that used for the solvent in the NaCl solutions). The Paramecia remained in the solutions 48 hours, were then taken out, counted, killed, and measured. This was done several times but as the results were in every case the same, only one series is reported here (see table VII).

TABLE VII

Dimensions of Paramecia that have remained 48 hours in various strengths of sodium chloride, compared with controls. Measurements are in microns and each is the average taken from 50 specimens. 100 specimens in each watch glass at beginning.

	NO. OF SPECIMENS AFTER 48 HOURS	LENGTH	WIDTH
Control	194	190.2	48.5
$\frac{N}{20}$ NaCl	220	173.5	49.0
$\frac{N}{30}$ NaCl	180	181.1	49.8
$\frac{N}{40}$ NaCl	210	188.4	49.1
$\frac{N}{50}$ NaCl	234	182.4	48.4

The table shows that these concentrations of NaCl have had no character stic effect either upon the rate of division or upon the size.

Only in the case of $\frac{N}{20}$ NaCl was there a slight indication of the modification of the rate of division, this concentration standing on the border line between the injurious and non-injurious solutions. In table VII, 100 Paramecia in $\frac{N}{20}$ NaCl had increased to 220, while an equal number in the control had increased to but 194. In another case, 50 specimens left in $\frac{N}{20}$ NaCl for 48 hours had increased to but 64, while in the corresponding control 109 had been produced.

Direct Measurement of Growth after Fission

Growth in solutions of NaCl was next measured directly by placing in the solutions to be examined specimens that had just divided, as described on page 491.

$\frac{N}{10}$ NaCl in hay infusion. The measurements in hay infusion to which NaCl had been added, and in the same infusion without the salt, are given in table VIII.

TABLE VIII

Comparative measurements in microns of growing Paramecia in $\frac{N}{10}$ NaCl dissolved in hay infusion, and in hay infusion.

AGE	CONTROL			$\frac{N}{10}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
5	152.9	50.4	18	153.2	37.2	16
10	162.5	52.2	22	161.7	37.2	11
20	166.9	51.7	14	162.1	39.1	15
40	186.9	49.6	19	141.5	42.0	11
65	182.4	50.1	18	142.6	41.4	11

For the first 10 minutes after separation, the halves in the salt solution increased in length at the same rate as the controls, then the length decreased rapidly for 40 minutes, when the Paramecia in the salt had become shorter than they were at the time of sepa-

ration. All those in the salt were dead at 60 minutes; many had died earlier; the latter, of course, were not measured. The width of those in the salt decreased very rapidly in the first 5 minutes, then remained the same up to 60 minutes, when the animals died.

$\frac{N}{20}$ *NaCl in hay infusion.* Table IX gives the measurements for growth when $\frac{N}{20}$ NaCl was added to hay infusion.

TABLE IX

Measurements of growth in $\frac{N}{20}$ NaCl *in hay infusion.*

AGE	CONTROL			$\frac{N}{20}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	144.3	52.2	7			
5	156.3	49.5	13	171.6	43.8	12
15	162.9	50.1	10	163.8	40.8	10
30	171.0	46.5	8	168.0	45.6	10
60	189.3	45.3	12	174.9	43.5	10
90	187.8	45.6	14	156.9	45.6	14
						6 alive
						8 dead
Hours						
6	All alive		50	44 alive		50
				6 dead		
25	198.6	58.5	(50 to) 56	181.8	52.1	
	All alive					
				23 alive		
				16 dead		11 Meas.
40	All alive		(56 to) 65	39 dead		50

For the first 20 minutes in this solution, the growth in length was normal. The width, however, showed a marked decrease, similar to that shown in the $\frac{N}{10}$ NaCl. At the end of 30 minutes, however, the Paramecia were normal in both length and width. At 60 and 90 minutes those specimens in the salt were shorter than the controls. After 90 minutes the Paramecia in the salt showed great variations in growth. Shortly after the age of 90 minutes specimens began to die, although some were alive at 30 hours. At the age of 40 hours none were alive in the salt, while

the controls had increased from 50 specimens to 65. At 25 hours, some of each were killed and measured. The controls measured 200.7 ×59.4 microns; those in the salt, 181.2 × 53.4 microns. This experiment shows that $\frac{N}{20}$ NaCl has a definite effect upon growth, inhibiting it after 60 minutes and finally causing death. It also shows that there is a great variation in the resistance of the animals to this strength of the chemical, some dying in 6 hours while other live 30 hours.

Growth in $\frac{N}{30}$ *NaCl.* In $\frac{N}{30}$ NaCl we have a strength of the chemical which may or may not affect growing Paramecia, depending on the conditions. In one of my experiments, there was apparently no effect of the salt upon the growing Paramecia at any time. Those in the salt grew to the same size as the controls (see table X).

TABLE X

Measurements of growth in $\frac{N}{30}$ NaCl.

AGE	CONTROL			$\frac{N}{30}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	144.3	52.2	8			
90	186.3	44.7	10	186.3	44.7	10
Hours						
24	192.3	66.	10	186.6	60.9	10

In another experiment, under what seemed exactly the same conditions as before, the $\frac{N}{30}$ NaCl had a slight inhibiting effect upon the growth. The measurements are given in table XI.

At the ages of 90 minutes, 5 hours, and 24 hours, those in the salt were shorter than the controls; sufficient numbers have been taken to give the figures some weight. I have worked out the probable errors for the averges, and find that, taking three times the probable error of each and substracting from the greater and adding to the less, in each case we still have significant differences, greater at 90 minutes and 5 hours than at 24 hours. Thus it seems from the analysis of the figures that there is in this case an

actual, though slight, inhibition of growth due to the $\frac{N}{30}$ NaCl. Thus $\frac{N}{30}$ is on the border line between a strength that will and one that will not affect growth in Paramecia.

$\frac{N}{40}$ *and* $\frac{N}{50}$ *NaCl in hay infusion.* Neither of these concentrations has any characteristic effect on the growth. See tables XII and XIII.

Rôle of Osmotic Pressure in Effects of Sodium Chloride

We may now ask whether these effects of NaCl are due to the osmotic pressure of the sodium chloride in solution acting on the Paramecia, or to the direct chemical effects of the substance, or to both.

By means of freezing point determinations,[5] we find that the osmotic pressure of ordinary hay infusion is .44 atmospheres. In table XIV is given the osmotic pressure of the different solutions of sodium chloride which we have used.

By examination of the table we find that an increase in the osmotic pressure from .44 atmospheres (that of hay infusion), to 1.841 atmoshperes (that of $\frac{N}{30}$ NaCl in hay infusion), has only a slight effect on the growth. Growth that was almost normal took place in $\frac{N}{30}$ NaCl in hay infusion. But when we increase to an osmotic pressure of 2.528 atmospheres, that of $\frac{N}{20}$ NaCl in hay infusion, there is a marked effect on the growth, which is even more marked at the pressure of 4.546 (that of $\frac{N}{10}$ NaCl.) This would seem to indicate then that an increase in osmotic pressure had an injurious effect on growing Paramecia. To give

[5] The osmotic pressure of the hay infusion has been found by the lowering of the freezing point method. When the lowering of the freezing point has been determined by the Beckman apparatus, the osmotic pressure is computed from the following forumula:

$$\text{O. P.} = \Delta \times \frac{22.3}{1.86^\circ}$$

Where Δ is the lowering of the freezing point, 22.3 is the osmotic pressure of a weight normal solution, and 1.86° is the lowering of the freezing point caused by a weight normal solution. The freezing point of a sample of hay infusion such as was known to be favorable to the growth of Paramecium was found to be .036 below that of pure distilled water, and by applying the above forumla the osmotic pressure of the hay infusion was .44 atmospheres.

TABLE XI

Measurements of growth in $\frac{N}{30}$ NaCl.

AGE	CONTROL			$\frac{N}{30}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.0	50	10			
90	198.3 ± .987	42.1	20	176.55±1.230	45.1	20
Hours						
5	119.59±1.476	41.6	34	190.80± .897	43.8	35
24	206.17±1.758	55.5	18	192.34±2.136	57.2	18

TABLE XII

Measurements in growth of $\frac{N}{40}$ NaCl

AGE	CONTROL			SALT		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.8	53.4	9			
30	174.0	49.5	9	174.0	46.8	10
90	201.9	45.9	17	201.0	45.3	17
Hours						
5	200.7	44.4	20	196.2	46.5	21
24	219.4	63.6	14	224.8	64.8	15

TABLE XIII

Measurements of growth in 50 NaCl

AGE	CONTROL			SALT		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	131.1	49.8	7			
5	145.2	47.7	7	145.2	48.9	7
15	159.3	47.4	9	160.5	46.8	10
30	168.9	45.0	11	171.3	43.2	11
90	194.4	45.6	19	189.3	46.8	19
Hours						
5	194.7	42.3	20	193.8	43.8	20
24	198.4	60.0	22	202.9	58.2	20

further light upon the part played by osmotic pressure, a .079 N solution of cane sugar was made by dissolving 2.681 grams of sugar, which had been rendered free from all impurities, especially sodium chloride, in 100 grams of pure water. This solution has an osmotic pressure of 2.087 atmospheres and is isotonic with a $\frac{N}{20}$ solution of NaCl in distilled water.[6] Halves of Paramecia just divided were used, one-half put in the sugar, the other half in the NaCl solution, both being carefully washed free of any hay infusion. Now if the injurious action upon Paramecia which we have observed is due to osmotic pressure alone, we would expect the two solutions to have the same effect upon the animals in this experiment. The results are shown in Table XV.

Up to the age of 90 minutes, the specimens in the sugar did not grow as large as normal, but did grow faster than those in the salt. At 5 hours, those in the sugar were slightly larger than at 90 minutes, but still smaller than normal, while those in the NaCl were dead. At 24 hours those in the sugar were not as large as at 5 hours, but it must be noted that there was no food in the sugar solution. The animals in the sugar were alive, but small, however, at 48 hours. This experiment then shows that osmotic pressure has an effect upon growing Paramecia, but that the injury caused by the sodium chloride is not due entirely to the osmotic pressure of the solution, but is also due to some other factor. The effects caused by the action of the sodium chloride then, are due to the osmotic pressure exerted by the sodium chloride plus the direct action of the salt upon the protoplasm of the Paramecium. So far as is known the action of the sugar is due entirely to its osmotic pressure.

To see the effect of even higher osmotic pressures upon Paramecium, growth in a $\frac{N}{10}$ NaCl in hay infusion was compared with the growth in an isotonic solution of sugar, *i.e.*, a .16 N solution in hay infusion. Growing Paramecia were used, and it was found that the Paramecia died in both of these solutions at about the same time, 10 to 15 minutes. The inability of the Paramecia

[6] The measurements for the osmotic pressure of sugar solutions are taken from some unpublished work of Prof. H. N. Morse of the Johns Hopkins Chemical Laboratory.

TABLE XIV.

The osmotic pressures in atmospheres of different strengths of NaCl at 20° C. dissolved in distilled water and in hay infusion. The osmotic pressure of hay infusion is assumed to be .441 atmospheres.

STRENGTH OF NaCl	O. P. OF NaCl DISSOLVED IN DISTILLED WATER	O. P. OF NaCl DISSOLVED IN HAY INFUSION
$\frac{N}{10}$	4.105	4.546
$\frac{N}{20}$	2.087	2.528
$\frac{N}{30}$	1.40	1.841
$\frac{N}{50}$	.870	.....
$\frac{N}{70}$	.658	.....
$\frac{N}{100}$	.432	.....

TABLE XV.

Comparative measurements of in microns Paramecia in $\frac{N}{20}$ NaCl in distilled water, and in .079 N sugar in distilled water.

AGE	GROWTH IN $\frac{N}{10}$ NaCl IN DISTILLED WATER			GROWTH IN .079 N SUGAR IN DISTILLED WATER		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	140.7	50.4	10	140.7	50.4	10
10	142.5	42.0	10	158.4	45.0	9
30	164.4	42.7	12	165.0	43.2	12
90	163.5	45.3	13	173.1	47.0	15
Hours						
5	All dead	....	10	175.5	50.1	10
24	All dead	....	..	154.2	48.6	11
48		....	..	Alive	....	10

TABLE XVI.

Table showing different effects of $\frac{N}{20}$ NaCl upon adult and young Paramecia.

AFTER HOURS	ADULT, 32 SPECIMENS		YOUNG, 32 SPECIMENS	
6	*Alive*	*Dead*	*Alive*	*Dead*
12	32	..	19	13
40	32	..	4	28
	28	4	3	29

[7] The data for osmotic pressure given here have been compiled from the formulæ given on pages 41–44 in "The Rôle of Diffusion and Osmotic Pressure in Plants," by B. E. Livingston, Chicago, 1903.

to grow in these solutions, which have a large amount of the chemical present in them, is probably due as much to the extraction of water from the cells as to the direct action of the chemical upon the life activities.

Comparative Resistance of Adult and Young Paramecia to $\frac{N}{20}$ NaCl

Early in the work it was noticed that adult Paramecia put into a $\frac{N}{20}$ NaCl solution continued to live, the solution having apparently no effect upon them, while young Paramecia put into the solution just after division died, before the end of 30 hours (as set forth above). A careful test of the varying resistance in the two cases was made as follows: Into each of the two depressions of a single slide was put a few drops of $\frac{N}{20}$ NaCl solution. Into one was put an adult Paramecium, into the other a young one from a fission just completed; both the adult and the young coming from the same stock culture, so that the past history would be nearly the same. Thirty-two pairs such were arranged and their further fate observed. The results are shown in Table XVI.

This experiment and other data given above (page 500), shows that adult Paramecia may live normally and grow in a solution that sooner or later kills Paramecia if they are put into the solution just as the two halves separate at division.

Are Paramecia in Stages Preparatory to Division Like Adult or Like the Young in their Resistance to the Salt?

To test this the following experiment was tried: Paramecia which were preparing for division were selected. They were then grouped in pairs, those being put together which were in the same stage of preparation for fission. (This is very easy to do with much accuracy, since one accustomed to working with Paramecia can tell within a few minutes when a dividing Para-

mecium will separate). One of a pair was put into hay infusion, the other one of the pair into a $\frac{N}{20}$ NaCl solution in hay infusion. It was found that all the pairs separated within 15 minutes of each other, so that there is little room for error. They were allowed to grow for 24 hours, and then killed and measured separately. Of 20 sets of pairs the results are as follows:

Control; hay infusion; 17 dividing Paramecia gave 34 specimens measuring at the age of 24 hours: $213.264 \pm 1.566 \times 71.19$.

3 dividing Paramecia gave 6 normal specimens not measured.

$\frac{N}{20}$ NaCl in hay infusion; 17 dividing Paramecia, mates of those in control experiment, gave 34 specimens measuring at the age of 24 hours: $205.059 \pm 2.466 \times 71.64$.

3 dividing Paramecia gave 6, all died.

Taking into consideration the probable errors of the measurements, there is evidently no significant difference in size of the two sets. However, three of the dividing Paramecia in the sodium chloride, after separating to form 6 specimens, died, while the corresponding controls remained normal. Why six out of forty in the NaCl should die, while the rest throve as well as the controls, is difficult to explain. Possibly there is a critical early period in which injury may occur; if the animals survive this, they flourish. The experiment taken as a whole shows, however, that the chemical is not so injurious to the young if the parents have been subjected to it a short time before fission.

Acclimatization to NaCl

We have seen above that certain concentrations of NaCl decrease growth. It was therefore determined to cultivate Paramecia in one of these solutions, to see if a smaller race could be obtained from L_2, or if possible, this race might changeinto one of the other races described by Jennings.[8] It was very easy to acclimatize Paramecium to a $\frac{N}{10}$ NaCl solution. If introduced directly into such a solution, Paramecia, as we have seen, die in a few minutes. The method of procedure for producing acclimatization was as follows. Adult specimens were put into a

[8] Loc. cit., page 494.

$\frac{N}{20}$ NaCl solution for 24 hours, then for a like period into $\frac{N}{15}$, then into $\frac{N}{10}$ where they will now live and multiply.

Fifty Paramecia were put into hay infusion and 50 into $\frac{N}{10}$ NaCl in the same hay infusion. At the end of 24 hours, 50 of the control were put into fresh hay infusion, and 50 of those in the $\frac{N}{20}$ NaCl were put into $\frac{N}{10}$ NaCl. At the end of the 24 hours, there were, as the result of some division, 69 in the control culture, 64 in the salt. Forty-four of the control were put into fresh hay infusion, and the 64 in the salt were transferred to the $\frac{N}{10}$ NaCl. At the end of 48 hours, the control numbered 54; those in $\frac{N}{10}$ NaCl, 75. Fifty of each were killed and measured. The average measurements of the 50 controls were 188.7 × 64.8 microns, of the 50 in $\frac{N}{10}$ NaCl, 170.7 × 70.02 microns. The Paramecia in the salt, as well as those in the control, were large and active. There was plenty of bacterial food present in both, the only evident difference being the smaller size of those in the NaCl. After four days, the specimens in the salt solution stopped dividing, while some had divided in an abnormal way. In many the division was incomplete, so that two specimens remained connected. Nothing of the sort was seen in the controls, the latter continued to divide normally. After the Paramecia had been in the $\frac{N}{10}$ NaCl 6 days, many began to die. An attempt was made to save these by putting them into fresh hay infusion containing $\frac{N}{10}$ NaCl, but when a few were introduced into this from the old culture, they all died in one to two hours, while those remaining in the old $\frac{N}{10}$ NaCl solution continued to live for nearly 20 hours more. The controls remained normal and alive.

Thus it is easy to acclimatize Paramecia to $\frac{N}{10}$ NaCl for a short period, but it is more difficult to get them into perfect relation to their changed environment. The animals in the salt at the latter end of the experiment were evidently in a very unstable equilibrium with their environment. Possibly the effect was caused by the accumulation of the sodium chloride in the cells.

Summarizing the work with sodium chloride, we find that the addition to the culture medium of sufficient NaCl to make a $\frac{N}{10}$ or $\frac{N}{20}$ solution permits the young specimens to grow for a few minutes after fission, then inhibits growth, finally causing death.

$\frac{N}{30}$ NaCl is on the border line; it sometimes decreases growth, sometimes not, dependinig on the conditions. More dilute solutions do not effect growth. Young specimens are injured by $\frac{N}{20}$ NaCl, while old ones are not. If the parents are subjected to this concentration for a short while before fission, the injury to the young is less. Paramecia can be temporarily acclimatized to a solution that would at first kill them, but after some days in such a solution fission becomes abnormal and ceases and the animals die. The injurious effects of sodium chloride are due partly to increase of osmotic pressure, partly to specific chemical action.

EFFECTS OF NICOTINE ON GROWTH

Having studied the effects of the comparatively innocuous substance, NaCl, I now undertook to examine the effects of nicotine ($C_{10}H_{14}N_2$). This alkaloid is so poisonous that only minute quantities are required to produce an effect; so minute that the osmotic pressure produced by them is clearly of no importance in the results. The effect of nicotine on single cells is of interest in view of its effect on man (see Lee, Langley, and others).

Effect of Nicotine upon Adult Paramecia

One hundred adult specimens of Paramecia taken at random from the stock culture were put into watch glasses containing known amounts of nicotine dissolved in hay infusion. These were allowed to remain for 50 hours. The results are given in table XVII.

From this table it is clear that strengths of nicotine from 1–1,000 up to 1–20,000 kill adult Paramecia in different times varying from 2 hours to 50 hours. Those in the 1–40,0000 solution and the more dilute solutions continued to live. In order to determine whether there was any effect upon the size a number of specimens were taken from these solutions, killed, and measured (see table XVIII.)

TABLE XVII

Effect of different strengths of nicotine upon Paramecia. 100 specimens each in watch glass at beginning of experiment.

PARTS OF NICOTENE IN PARTS OF HAY FUSION.	STARTED FEB. 1, 1909, 12:30 P. M.				
	Feb. 1, 2 P. M.	Feb. 1, 5. P M.	Feb. 1, 9 A. M.	Feb. 3, 10 A. M.	Feb. 3, 3 P. M.
1–1,000	All dead	.....	.....	.....	.....
1–1,250	All dead but 2	All dead	.....	.....	.....
1–1,666	All dead but 60	Few alive	All dead	.....	.....
1–2,000	All dead but 30	All dead	.....	.....	.....
1–2,500	50 dead	More dying	All dead	.....	.....
1–5,000	50 dead	More dying	10 alive	All dead	.....
1–10,000	All alive	All alive	Many dying	6 alive	All dead
1–20,000	All alive	All alive	Some dying	5 alive	3 alive
1–40,000	All alive	All alive	All alive	All alive	All alive
1–80,000	All alive	All alive	All alive	All alive	All alive
1–100,000	All alive	All alive	All alive	All alive	All alive
Control hay infusion	All alive	All alive	All alive	All alive	All alive

TABLE XVIII

Dimensions of Paramecia that have remained 50 hours in various strengths of nicotine compared with controls; measurements are in micorns, and each is the average taken from 50 specimens.

	LENGTH	WIDTH
At beginning of experiment	219.4	46.3
Control at end	260.7	67.5
1–40,000	213.3	63.4
1–80,000	210.2	64.0
1–100,000	214.5	63.4

It is seen that the Paramecia in the nicotine were about the same size at the end of the experiment as they were at the beginning. The controls, however, had increased in size from 219.4 × 46.3 microns to 260.7 × 67.5 microns. This would seem to indicate that those in the nicotine did not grow at all durng the period of the experiment.

Effect of Nicotine on Growing Paramecia

Growth in 1-2,500 nicotine. Growth in nicotine was measured directly in the way previously described (page 491). One of the two products of fission was placed in pure hay infusion, the other in hay infusion containing nicotine. In a solution of 1 part of nicotine to 2,500 of hay infusion, the results are as shown in table XIX.

TABLE XIX

Measurements of growth in 1-2,500 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.0	53.0	10			
5	159.7	57.7	12	155.4	59.2	12
10	164.1	58.2	11	157.8	62.4	10
20	172.1	56.2	11	156.5	69.8	11
30	169.0	57.6	9	163.5	65.1	10
60	191.7	52.6	9	160.2	68.7	9
Hours						
2	All alive		11	4 alive	7 dead	11
5	All alive		11	11 dead		11

TABLE XX

Measurements of growth in 1-5,000 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	132.6	53.4	10			
5	151.2	55.0	9	149.7	52.2	10
15	156.3	53.1	10	152.1	54.3	10
30	167.7	51.6	11	162.3	54.7	9
60	185.4	50.1	11	168.3	53.1	9
Hours						
9	197.7	52.5	11	175.2	49.2	7
24	212.1	62.1	25	174.9	48.4	25
				8 dead	10 alive	7 measured.
48	All alive		18	18 dead		18

At the end of five minutes, though there was some growth, those in the nicotine were not quite so large as the controls. At 30 minutes, they were still growing, but continued to be smaller than the controls. At 60 minutes, they began to decrease in size. At the end of 2 hours, of 11 specimens in the nicotine, 4 were alive and 7 dead. It is to be noticed that some growth took place in a solution of nicotine which later killed the organism.

Growth in 1–5,000 nicotine. In one part of nicotine to 5,000 of hay infusion, growth took place in the same general way as in the 1–2,5000 solution (see table XX).

The Paramecia grew in the nicotine, but were not at any time so large as the controls. At the age of 9 hours, those in the nicotine measured 175.2 × 49.2 microns, while the controls were 197.7 ×52.5. At the age of 24 hours, those in the nicotine were beginning to die, and at 48 hours, all those in the nicotine were dead.

Growth in 1–10,000 nicotine. In a 1–10,000 solution of nicotine, the Paramecia grew at about the same rate as those in the 1–5,000 (see table XXI), remaining somewhat behind the controls.

The difference is not great, but the results indicate a slight inhibition of growth caused by this amount of nicotine. All those in the nicotine were dead at 45 hours.

Growth in 1–20,000 nicotine. The results with 1–20,000 nicotine were variable (see table XXII).

Up to 5 hours, the growth was not evidently affected. At later periods the specimens in the nicotine were sometimes smaller, sometimes larger, than the controls. At 24, and 48 hours, they were distinctly smaller. Thus the effect of such a quantity of nicotine shows only after a considerable period.

Growth in 1–30,000 nicotine. In 1–30,000 nicotine, growth took place as in the control (see table XXIII).

At the end of 24 hours, the measurements in the nicotine were 193.2 × 63.9 microns, while the controls measured 187.5 × 68.7 microns. The difference is without significance; it is partly due to the fact that one of the controls had divided, the two small specimens so produced reducing the average length.

TABLE XXI

Measurements of growth in 1–10,000 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.8	53.7	10			
5	153.6	51.3	9	152.1	55.8	10
15	166.8	52.5	10	163.2	54.3	9
30	175.2	51.3	10	171.6	48.9	9
60	192.9	53.7	12	183.3	53.7	10
90	189.9	50.1	9	185.1	51.0	12
Hours						
5	180.9	45.6	9	170.7	52.5	8
24	186.0	55.8	5	177.6	49.8	5
45	All alive		17	17 dead		17

TABLE XXII

Measurements of growth in 1-20,000 nicotine

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	141.9	48.0	9			
90	187.8	43.5	8	185.1	45.9	11
Hours						
5	180.0	45.6	10	188.4	48.6	10
24	187.2	56.4	10	173.7	54.9	9
48	195.2	62.4	(10to12	185.2	53.2	8

TABLE XXIII

Measurements of growth in 1–30,000 nicotine

AGE	CONTROL			NICOTINE		
	Length	Width	No, of Specimen	Length	Width	No. of Specimens
Min.						
0	139.8	49.5	10			
90	167.4	46.2	10	168.9	45.3	10
Hours						
5	175.8	44.4	5	180.6	44.4	5
24	187.5	68.7	(12 to)13	193.2	63.9	12

Different Resistonce of Young and Adults to Certain Concentrations

Interesting results were reached in the effect of nicotine upon Paramecia of different ages. Nine adult specimens were put into a 1–5,000 solution of nicotine. Nine specimens just divided were put into the same solution. At the end of 2 hours, 5 of the adult specimens were dead, while only 2 of the young were dead. At 20 hours, all 9 adults were dead, while all but 2 young were alive. At the end of 30 hours, all the young were dead.

Some adult Paramecia were put into 1–20,000 nicotine. These were all dead at the end of two days. Some specimens just divided, put into the same strength of nicotine, grew to normal size and lived for 5 days, but did not divide.

Thus in these grades of nicotine the young Paramecia are more resistant to the chemical than adult specimens. In NaCl, on the other hand, as has been shown, the adult specimens are more resistant than the young specimens.

Summing up the results with nicotine, we find that a certain amount of growth takes place in the stronger solutions of nicotine, though these same solutions later kill the organism. This is true for solutions up to and including 1–10,000. In 1–20,000 nicotine solution there was a very slight retardation of growth at late stages. In the 1–30,000 solution the nicotine had no effect on the growth.

EFFECTS OF STRYCHNINE ON GROWTH

Strychinne has long been known to be a powerful poison for protoplasm. The work of Schulze, Binz, and others has shown its action on Protozoa, but there are no studies as to its effect upon growth in smaller quantities than lethal doses. Calkins and Lieb have shown that, in small quantities, strychnine increases the rate of division in Paramecium, but they did not study its effect upon growth and size. It will be interesting to determine whether there is increased size at the time when the animals show the increased rate of division, described by the authors mentioned. The general effects of this powerful poison upon cellular growth are likewise of interest.

Effects of Strychnine upon Adult Paramecia

A $\frac{N}{1000}$ solution of strychnine nitrate was made by dissolvin .0387 grams of that salt in 1,000 cc. of distilled water. This solu tion then contained about one part of strychnine nitrate in 2,50 of water. As this was the strongest solution used, other strength desired were made by further dilution of this with hay infusion

The 1–2,500 solution of strychnine killed Paramecia in a very few minutes. A 1–12,500 solution caused them to swell up and become vacoulated almost immediately, although they remained alive and moving for several hours. With more dilute solutions the effect is not so immediate or marked. To determine the effect of the more dilute solutions, watch glass preparations (as with NaCl and nicotine) were made. Strengths of strychnine up to 1–37,500 killed a great many of the Paramecia. In the 1–50,000 there were as many at the end of 48 hours as at the beginning. The normal rate of division was maintained in the 1–125,000 and more dilute solutions. There was no effect at any time upon the size (see table XXIV).

TABLE XXIV

Table showing rate of division of Paramecia in different strengths of strychnine and in hay infusion. 100 specimens in each watch glass at beginning.

STRENGTH OF SOLUTIONS USED	NUMBER OF SPECIMENS AT END
Control I	179
Control II	217
Control III	200
1–12,500	3
1–25,000	23
1–37,500	83
1–50,000	99
1–75,000	182
1–125,000	162
1–150,000	198
1–200,000	203
1–250,000	193

Growth of Paramecium in Strychnine after Fission

Growth in strychnine was measured directly in the way previously described (page 491).

Growth in 1–12,500 strychnine. For the first five minutes after separation, those in the alkaloid grew to the same length as the controls. At 15 minutes, those in the strychnine decreased in length until they were as short as they were at the time of fission, and remained so for 60 minutes, when all were dead. The width of those in the alkaloid increased very rapidly in the first 30 minutes (see table XXV).

TABLE XXXV

Measurements of growth in 1–12,500 strychnine.

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	133.8	54.0	6			
5	154.8	54.0	8	153.0	59.2	8
15	165.0	53.4	5	134.4	69.0	5
30	178.5	52.2	7	134.7	82.2	9
60	All alive		10	All dead		10

TABLE XXVI

Measurements of Growth in 1-50,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	134.1	51.9	7			
5	143.4	50.7	10	145.8	51.0	10
15	151.1	49.2	11	150.9	49.8	11
30	160.5	47.1	12	155.2	51.7	12
60	182.7	43.5	11	153.7	53.2	12
90	189.0	42.9	11	164.1	48.1	11
Hours						
2	All alive		10	Swollen and dying		10

TABLE XXVII

Measurements of growth in 1-75,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No of Specimens	Length	Width	No. of Specimens
Min.						
0	137.4	49.2	10			
5	149.4	48.0	9	150.3	49.2	9
15	159.3	48.6	10	157.8	49.2	10
30	165.6	43.8	10	161.4	47.7	10
90	191.4	40.5	10	171.0	46.8	10
Hours						
5	190.8	40.8	12	163.8	49.8	11
24	All alive		13	3 barely alive; 10 dead		13

Growth in 1–50,000 strychnine. Normal growth took place in a 1–50,000 solution of strychnine for the first 15 minutes (see table XXVI).

Further growth then took place, but was not so rapid as in the control. At the end of 2 hours those in the strychnine were dying.

Growth in 1–75,000 solution of strychnine. For the first 15 minutes after separation, the halves in the strychnine increased at the same rate as the control (see table XXVII).

Further growth took place, but was not so rapid as in the control. At 24 hours, of 13 specimens in the strychnine 10 were dead and 3 dying.

Growth in 1–100,000 strychnine. Normal growth took place in 1–100,000 strychnine up to the age of 30 minutes, the specimens in the alkaloid growing to the same size as in the control (see table XXVIII).

Growth then continued, but not so great in amount as in the control. At 5 hours, those in the strychnine were much shorter than the controls, but at 24 hours they had almost reached normal size again. At the age of 48 hours, the Paramecia in the strychnine had not grown as much as the controls; 14 specimens

in the strychnine had not divided and measured 181.2 × 44.1 microns, while 14 specimens in the control had increased by division to 33 and these measured 197.4 × 51.6 microns. Thus we find that in the 1–100,000 strychnine normal growth takes place up to the age of 30 minutes, then further growth ensues, but is not so great in amount and the specimens do not become as large at 48 hours as in the control. Division is also stopped in this strength of strychnine.

Growth in 1–125,000 strychnine. This concentration has no effect on the growth (see table XXIX.)

TABLE XXVIII

Measurements of growth in 1-100,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No of Specimens	Length	Width	No. of Specimens
Min.						
0	142.1	52.8	8			
5	145.8	50.7	10	146.4	50.1	10
15	149.7	51.9	9	156.6	52.5	10
30	162.3	46.2	11	161.1	48.3	12
90	191.4	45.6	9	179.4	51.9	9
Hours						
5	183.5	47.1	11	153.3	47.4	12
24	199.3	70.5	20	196.3	49.5	22
48	197.4	51.6	(14 to) 33	181.2	44.1	14

TABLE XXIX

Measurements of growth in 1-125,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.2	49.8	8			
30	166.2	45.6	9	169.8	46.8	6
90	188.1	43.2	10	188.1	45.6	10
Hours						
5	191.5	46.4	13	194.8	48.0	15
24	206.3	52.6	17	206.1	59.7	18
48	220.5	55.5	(5 to) 10	212.7	54.6	(5 to) 8

Growth in 1–200,000 and 1–250,000 strychnine. It was noted above that certain investigators had found that minute quantities of strychnine increased the division rate in Paramecium; in order to determine whether increased size accompanied this increased rate of division, more dilute strengths were used. In both 1–200,000 and 1–250,000, growth was exactly the same as in the controls (see tables XXX and XXXI). There was no evidence at any time of either increased size or increased rate of division.

Comparative Resistance of Adult and Young Paramecia to Strychnine.

Twenty adult Paramecia and 20 young Paramecia from the same stock culture were put into 1–25,000 strychnine solution.

RESULT

	ADULT; 20 SPECIMENS		YOUNG; 20 SPECIMENS
After Hours	*Alive*	*Dead*	
2	18	2	Some swollen
5	18	2	All dead
12	9	9	All dead
24	2	18	All dead

The same experiment was again tried using 1–50,000 strychnine in place of the 1–25,000. The results follow.

	ADULT; 20 SPECIMENS	YOUNG; 20 SPECIMENS
After Hours		
6	All alive	Few swollen
6	All alive	Dying
11	All alive	All dead
24	13 dying, 7 dead	All dead

It is evident from these experiments that adult Paramecia have a much greater resistance than young specimens to the stronger solutions of strychnine.

Summarizing the effects of strychnine, we find that when specimens just after fission are introduced into hay infusion containing strychnine at concentrations of from 1–12,500 to 1–75,000 a

TABLE XXX

Measurements of growth in 1-200,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Hours						
0	145.0	50.0	10			
5	195.9	39.9	10	199.2	45.0	9
24	196.1	55.1	20	193.3	59.5	20
48	201.3	48.3	(29 to) 39	205.9	52.5	(29 to) 30

TABLE XXXI

Measurements of growth in 1-250,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Hours						
0	145.0	50.0	10			
24	193.8	50.1	10	191.1	55.5	10
48	196.4	44.3	(20 to) 32	203.8	48.7	(20 to) 27

TABLE XXXII

Measurements of growth in 5 per cent alcohol

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.3	44.4	10			
5	146.7	45.9	10	137.7	44.7	9
30	163.2	39.3	8	146.4	45.3	10
90	165.8	39.5	11	153.0	44.4	9
Hours						
3	176.1	39.0	11	156.9	53.7	10
5	All alive		15	Dead 15 sp.		15

certain amount of growth takes place in the first few minutes, then the animals die. In strychnine at 1–100,000, growth is almost normal in amount. No evidence appeared of increased size or increased rate of fission due to strychnine in any strength.

Adult Paramecia are more resistant than young specimens to the stronger solutions.

Effects of Alcohol on Growth

Introductory. So much has been written in the last few years, and such diverse results reached on the effect of alcohol upon higher animals that it will be interesting to study the effects of alcohol upon growth in single cells. Calkins and Lieb,[9] in 1902, showed that "alcohol had no effect upon Paramecium taken in too weak doses and too powerful an effect when taken in over strong doses," also "when a medium dose was given, *i.e.*, one part of alcohol in 2,500 of hay infusion, the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series."

Woodruff,[10] in 1908, found that alcohol increased the rate of division at certain periods in the life cycle of Paramecium and decreased it at others. He also found that the increased rate of division was not lasting, but that doubling the amount of alcohol again caused a rapid cell division for a limited period.

It will be of value, then, to see what effect different amounts of alcohol will have upon growing Paramecia, and whether minute quantities will cause increased size or increased rate of division.

Effect of Alcohol on Growing Paramecia

Growth in 5 per cent solution of alcohol in hay infusion. Young Paramecia just after separation were used, in the way described on page 491. Some growth took place in a 5 per cent solution of alcohol in hay infusion, but the animals did not grow so fast at any time as the controls.[11] All those in the alcohol were dead at the end of 5 hours (see table XXXII).

Growth in 3 per cent alcohol. A certain amount of growth took place in 3 per cent alcohol, but the specimens in the alcohol did

[9] Loc. cit., page 364.

[10] Loc. cit., page 85.

[11] The slides containing the alcohol cultures of Paramecia were kept in a separate chamber from the the controls. In the moist chamber containing the alcohol cultures, the bottom was covered by a 5% solution of alcohol and in this way loss of alcohol by evaporation from the culture drops was prevented. The control cultures were of course kept in water vapor alone. In the other strengths of alcohol studied, similar precautions were taken.

TABLE XXXIII

Measurements of growth in 3 per cent alcohol

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.2	45.6	10			
30	162.0	39.3	10	153.9	39.6	10
90	187.0	38.2	18	173.1	39.3	16
Hours						
5	191.7	37.2	11	185.7	48.0	10
12	All alive		20	All dead		20

not at any time grow as fast in the alcohol as in the control (see table XXXIII). At 12 hours, all those in the alcohol were dead.

Growth in 2 per cent alcohol. At the age of 90 minutes, Paramecia had grown to normal length, but were not so thick as the controls. At 5 hours, they were slightly shorter in length and much thinner than the controls (see table XXXIV). At 24 hours, those in the alcohol measured 196.1 × 56.5 microns, and had not divided at all, while the controls had increased from 22 to 42 specimens. Thus in 2 per cent alcohol animals had grown to about normal size, but did not divide. The effect of the alcohol is shown mainly on the inhibition of division.

Growth in 1 per cent alcohol. At the end of 90 minutes, Paramecia growing in 1 per cent alcohol were slightly larger than the controls. At 5 hours and at 24 hours those in the alcohol had grown to the same size as the controls. Thus 1 per cent alcohol has no effect upon the growth (see table XXXV).

Growth in 1–500 alcohol. As was mentioned above, it has been found by certain investigators that minute quantities of alcohol cause increased rate of division. In order to see if increased size accompanied this greater division rate, the effects of more dilute solutions of alcohol were tried upon growing Paramecia.

Paramecia grew to exactly the same size in the 1–500 alcohol as in the control (see table XXXVI).

Growth in 1–2,500 alcohol. In a solution of 1 part alcohol in 2,500 of hay infusion, Paramecia grew to the age of 5 hours, at the

TABLE XXXIV.

Measurements of growth in 2 per cent alcohol.

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	191.0	52.3	9	192.0	43.2	9
Hours						
5	190.2	45.7	9	186.3	36.6	9
24	22 specimens increased to 42		(22 to) 42	196.1	56.5	22

TABEL XXXV.

Measurements of growth in 1 per cent alcohol.

AGE	CONTROL.			ALCOHOL.		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	189.0	42.6	9	197.7	43.3	9
Hours						
5	202.2	44.7	13	205.6	40.8	15
24	210.6	67.7	34	213.6	69.6	33

TABLE XXXVI.

Measurements of growth in 1-500 alcohol

AGE.	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	185.2	38.4	18	186.6	37.9	16
Hours						
5	190.5	38.4	21	187.4	41.1	21
24	204.7	64.8	29	202.0	63.1	30

same rate as in the control (see table XXXVII). At 24 hours, however, those in the alcohol were 6.2 microns longer than the controls. If the probable errors of the length are inspected, this

TABLE XXXVII

Measurements of growth on 1-2,500 alcohol.

AGE	CONTROL			ALCOHOL		
	Length	Width	NO. OF SPECIMENS	Length	Width	NO. OF SPECIMENS
Min						
0	133.8	40.5	10			
30	161.0	40.3	9	156.6	38.1	10
90	171.0	35.1	10	168.3	37.8	10
Hours						
5	179.7	40.5	10	178.6	38.7	9
24	199.7	63.6	21	205.9	65.5	20
	1.479			2.094		

difference becomes insignificant. So the result would indicate that the alcohol had not increased the growth above the normal amount.

Growth in hay infusion of Paramecia treated for 30 minutes after fission with 7½ per cent alcohol. To see if treatment with a strong solution of alcohol for a short period would have any lasting effect upon the animals when transferred to normal conditions, or whether the animals would be inhibited in growth for the actual time in which they were in the alcohol, the following experiment was tried: Paramecia just after fission were put into 7½ per cent alcohol and allowed to remain. At the age of 5 minutes, and 30 minutes, they were shorter than they were at the time of separation. They were also swollen and vacuolated and motionless. Water evidently had been extracted from the Paramecia by the strong alcohol (see table XXXVIII).

At the end of 30 minutes the Paramecia in the alcohol were taken out and put into hay infusion. Fifteen minutes after being taken out of the alcohol or at the age of 45 minutes, they had increased from 121.8 microns (the size at which they were transferred from the 7½ per cent alcohol to the hay infusion), to 147.9 microns, being then sightly larger than at the time of fission. No further growth took place in the Paramecia that had been treated for 30 minutes with the alcohol until shortly after 5 hours. At that time the Parmecia began to increase in size, and

TABLE XXXVIII

Measurements of growth of Paramecia treated with 7½ per cent alcohol for 30 minutes after separation and then placed in hay infusion

AGE	CONTROL Paramecia in Hay Infusion			EXPERIMENT			
	Length	Width	No. of Specimens		Length	Width	No. of Specimens
Min.							
0	142.2	44.4	10	in 7 ½ per cent alcohol			
5	144.6	45.0	9	in 7½ per cent alcohol	123.3	48.9	10
30	150.3	42.0	10	in 7½ per cent alcohol	121.8	54.0	8
45	175.5	41.0	9	30 minutes in alcohol, 15 minutes in hay infusion	147.9	41.7	10
60	187.9	42.7	8	30 minutes in alcohol, 30 minutes in hay infusion	144.7	46.6	9
Hrs.							
2	184.5	38.6	16	30 minutes in alcohol, 90 minutes in hay infusion	148.4	39.4	13
5	171.5	42.3	11	30 minutes in alcohol, 4½ hours in hay infusion	137.4	42.8	11
24	177.5	49.5	16	30 minutes in alcohol, 23½ hours in hay infusion.	160.6	51.7	13
48	187.3	53.7	(6 to)9	30 minutes in alcohol, 47½ hours in hay infusion	184.9	59.4	(6 to) 11

at 24 hours they measured 160.6 × 51.7 microns. They were at that time shorter than the controls, which had not been treated at the beginning with the alcohol. At the age of 48 hours, the control had increased from 6 to 9 specimens and measured 187.3 × 53.7 microns, while those that had been treated with the alcohol for 30 minutes after fission, and then placed in hay infusion, had increased from 6 to 11 specimens, and measured 184.9 × 59.4. At 48 hours the Paramecia had entirely recovered from the effects of the initial treatment with alcohol and had regained their normal size.

Thus we find that $7\frac{1}{2}$ per cent alcohol inhibits growth completely, and that the inhibitory effect lasts for some hours after the animals have been removed from it. But finally the inherent tendency to grow overcomes the effects of the alcohol, and the specimens regain their normal size and rate of growth.

Resistance of Adult and Young to Alcohol

There is no such marked difference in the resistance of a young and adult Paramecia to strong solutions of alcohol as we find with the other chemicals. In a 5 per cent solution young and adults die in about the same time (2 to 3 hours). In a 3 per cent solution the adults are slightly more resistant than the young, living in the solution about 12 hours, while the young die in about 5 hours.

The relation of alcohol to growth casts some light in the rôle of osmotic pressure in producing the effects of chemicals on growth. We find that nearly normal growth takes place in 2 per cent solution of alcohol; this has an osmotic pressure of 8.24 atmospheres, while Paramecia die quickly in a $\frac{N}{10}$ solution of NaCl, with an osmotic pressure of but 4.55 atmospheres. This indicates that changes in osmotic pressure have only a small part in the effects of these chemicals upon growth.

Thus the effects of alcohol on growth in Paramecium are in a general way similar to the effects of the other chemicals studied. The stronger solutions, 5 per cent and 3 per cent, inhibit growth somewhat, and the animals finally die. Normal growth takes place in 1 per cent alcohol. No evidence was found that minute quantities of alcohol increase growth. The adult Paramecia are slightly more resistant to alcohol than the young.

Effect of Food on Growing Paramecia

At almost every step in these experiments, the question arose as to what part the presence or absence of food plays in the growth of Paramecium. We have seen that during the first 90 minutes, growth took place very fast. To what is this rapid growth due? Is it due to ingested matter or to imbibition of water? Growing Paramecia were put into hay infusion containing India ink, and it was found that no particles of the India ink were ingested into the body of the animal until about 30 minutes after fission. Thus the marked increase in size which takes place in the first 30 minutes in a growing Paramecium cannot be due to ingested matter of any sort. The only other explanation for the increase in size is the imbibition of water.

To determine directly the effect of food upon growth in the early stages, the growth of Paramecium in tap water containing rather little bacterial food was compared with the growth in fresh hay infusion. Halves of divided specimens were put into hay infusion, the other halves of the same specimens into tap water which had been boiled and then aerated. At the end of one hour, 35 specimens in the hay infusion measured 173.1 × 40.9 microns, 35 in the tap water 171.4 × 40.6 microns. We find that the same amount of growth has taken place in both.

The growth in tap water as prepared above was then compared with that hay infusion in which there was dense bacterial growth. Specimens just after fission were used and kept in the different culture fluids one hour. At the end of that time, the Paramecia in the hay infusion with plenty of bacterial food present measured 169.8 × 42.0 microns (10 specimens), while those in the tap water with practically no bacterial food grew to about the same size, 171.3 × 42.3. From this experiment and the experiment before, growth to the age of 60 minutes would seem to be independent of the amount of food present, as the same amount of growth takes place in the tap water with no food present as in the hay infusion with the large amount of bacteria.

To see what effect food will have upon growing Paramecia for longer periods of time the following experiment was performed:

Some hay infusion was allowed to stand in a warm room until it became turbid from the growth of bacteria. Part of this was then filtered through a new Pasteur-Chamberlain bougie. The filtrate was thus rendered free of all bacteria and was also exactly the same, chemically and otherwise, as the hay infusion with the bacteria in it before filtering, except for the absence of the bacteria. Halves of dividing Paramecia were put into these two solutions, one with bacteria, the other without. At the end of 90 minutes, there was, as was to be expected, no difference in size. At the age of 5 hours, the Paramecia in the hay infusion with the bacterial food were shorter and thicker and measured 200.1 × 60.5 microns (14 specimens), while those growing in the media without bacteria were longer and thinner; 11 of these measured 210.9 × 45.0 microns. At the age of 24 hours the presence of food has had a marked effect. Twenty-eight specimens in the hay infusion with the bacteria had increased by division to 49 specimens, and these measured 193.5 × 62.2 microns, while the 28 specimens in the medium without food had not divided at all and measured 205.2 × 53.4 microns. It must be said here, however, that some growth of bacteria had taken place in the solution which was sterile in the beginning, as it is impossible to wash Paramecia absolutely free from adhering bacteria. Yet at 24 hours, there was evidently an abundance of bacteria in the one medium, and almost none in the other.

Thus in the early stages of growth up to the age of about 90 minutes the presence or absence of food material in the medium has no effect on the size of the growing animals. As we have seen earlier, the greatest amount of growth takes place in the first 90 minutes and our experiments indicate that this increase in size is due mainly to imbibition of water. From then on, the presence of food in the culture fluid has an effect on the size to which the Paramecia will attain. With plenty of food present the animals grow shorter and thicker, and divide sooner, than those kept in a medium with less food.

It is to be noted that in the experimental work in the general effects of the different chemicals studied, the question of bacterial food does not enter in the interpretation of the results. It was

found that in no case did the presence of any of the chemicals in the hay infusion stop the normal growth of the bacteria, the culture medium with the chemical in it becoming turbid with bacteria at about the same time as the control hay infusion. For this reason there will be about the same amount of food in one culture medium as the other, and the question of food affecting the general results will be negligible.

SUMMARY AND DISCUSSION

1. The investigations do not show that any of the substances studied have what could be called a specific or characteristic effect on growth. When very weak they have no effect whatever. In greater concentrations, all retard the later stages of growth, at the same time manifestly interfering with the other vital processes of the organism. When still stronger even the early stages of growth are impeded or prevented; in such cases the organism is quickly killed by the chemical. In no case is the growth affected without other injury to the organism. On the whole it appears clear that the effects on growth are secondary; they are consequences of the interference of the chemical with the other vital processes of the animal, and do not appear unless such interference exists. Essentially the same effects on growth appear whether the interference with other vital processes is due merely to the absence of any salts in the medium, to the presence of undue quantites of such a common substance as sodium chloride, or of minute quantites of such poisons as nicotine, alcohol or strychnine. None of the substances studied, whatever the amount present, has a tendency to increase the normal rate or amount of growth (although the presence of a small amount of sodium chloride permits this normal growth to occur, when it otherwise would do not so).

2. The early stages of growth show in certain respects a remarkable independence of the surrounding medium. In many cases, as we have seen, a certain amount of growth takes place under conditions which later destroy the organism. At fission the organism seems to have a certain potential of growth, due largely to

internal conditions; it has a strong tendency to grow in a perfectly definite way, at a definite rate, the rate giving a curve of a definite form. It grows for a time in this way in spite of the almost complete absence of the salts that are necessary for its continued existence, and in spite of the presence of actively injurious chemicals, which in a short time kill the organism. Whatever growth occurs tends to follow the normal growth curve. The substances within the organism, plus water, seem all that is necessary for this process, and it persists for a time in spite of positively injurious external conditions.

No evidence was found that a race of a given typical size can be transformed by any of the chemicals studied into a larger or smaller race. Their effects on growth seem due to interference with the vital processes, resulting in pathological conditions in other respects as well as in growth. Continued action of the chemicals that interfere with growth usually sooner or later cause death. The causes of the observed temporary changes in size in a given race under differing cultural conditions are probably to be sought for in variations in the nutritive and other conditions of the normal environment, particularly in conditions that affect the rate of fission.

3. On the precise nature of the deleterious action on growth in the case of the different chemicals the investigations gave little light. In the case of sodium chloride it appears possible, as we have seen, that a part of the injurious action is due to osmotic pressure, while a part is not. But in the case of nicotine and strychnine the minute quantites employed show that the osmotic pressure plays no part, yet these chemicals produce essentially the same effects on the growth as do undue amounts of sodium chloride. In the case of alcohol the growth occurs in solutions having a much higher osmotic pressure than the deleterious solutions of sodium chloride. Thus all the facts taken together seem to indicate that disturbance in osmotic relations plays little part in producing the effects on growth, even in the case of such substances as sodium chloride.

4. As to the processes in growth itself, it appears clear that the increase in size in early stages, up to 60 to 90 minutes (at

which time half the growth in length has occurred), is due almost solely to the imbibition of water. Up to this time growth occurs in much the same way whether there are food substances present in the water or not; as we have seen, it takes place when all the solid substances are removed from the fluid by filtering, or even in pure distilled water containing a little sodium chloride. After about ninety minutes the presence or absence of bacterial food in the medium has a noticeable effect on the growth. If plenty of bacterial food is present the animals are thicker, but do not grow so long as with little food; this is owing to the fact that where food is abundant, fission takes place more frequently. The animals may, however, reach the normal length in a medium containing almost no food; we have seen that this occurred in distilled water containing a little NaCl. But in such cases fission does not occur; this growth by mere imbibition of fluid can manifestly not continue for more than one generation.

5. In investigating the different resistance of young and adult Paramecia toward the stronger solutions of the chemicals, it was found that adult Paramecia were much more resistant than very young animals toward sodium chloride, strychnine, and alcohol, while the reverse was true with nicotine, the young being more resistant than the adult. It would be naturally expected from the work done on higher animals that the adult would be more resistant. The reversal of the effects found in nicotine are probably due to some specific effects of the nicotine which are not present in the other chemicals.

6. Acclimatization of Paramecium to strengths of sodium chloride, which would kill the animals if introduced directly, was found to be easy, but this acclimatization was not permanent and the animals finally died. In acclimatizing Paramecia to a $\frac{N}{10}$ NaCl solution the ratios of the different salts within the cell are probably changed to such a degree that it takes a long time for the cell to regain its equilibrium so as to continue its normal metabolic processes. Acclimatization of Paramecia to the other chemicals was not attempted.

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OLFACTORY REACTIONS IN FISHES

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The fact that the olfactory apparatus, both peripheral and central, is very well developed in most fishes has led many morphologists to ascribe to these animals a keen sense of smell; but this opinion has been unsupported by physiological evidence, for up to the present time investigators of the subject have not been able to demonstrate any form of stimulation or reaction characteristic of this apparatus in water-inhabiting vertebrates. The observations of Aronsohn ('84, p. 164), that a goldfish, which ordinarily will eat ant pupæ with avidity, will not take these pupæ after they have been smeared with a little oil of cloves, are not conclusive evidence that the fish scents the oil, for it is entirely possible that this oil merely irritates the skin of the fish's snout and does not stimulate the olfactory apparatus at all. Nor is the discovery made by Steiner ('88, p. 47), that the spontaneous appropriation of food by Scyllium ceases on the removal of the cerebral lobes or simply on cutting the connections between these lobes and the olfactory bulbs, satisfactory evidence that the olfactory apparatus in these fishes is an organ of smell rather than a receptor for taste or some closely allied sense. Nagel ('94, p. 184) noted that the front portion of the head of Barbus was as sensitive to sapid substances after the olfactory tracts had been cut as before that operation, and Sheldon ('09, p. 291), who has studied the dogfish with great fulness, demonstrated that the decided sensitiveness of the nostrils of this fish to weak solutions of oil of cloves, pennyroyal, thyme, etc., was not influenced by severing the olfactory crura, but disappeared on cutting the combined maxillary and mandibular branches of the trigeminal nerve.

Evidently the nostrils of fishes, like those of the higher vertebrates, are innervated by fibers from the trigeminal nerve, and it is this nervous mechanism rather than the olfactory apparatus that is stimulated by the substances that have ordinarily been applied by experimenters. In fact, so far as the olfactory apparatus of the fishes and amphibians is concerned, we must agree with Nagel ('94, p. 61) that no one thus far has discovered anything positive concerning its function. It is, therefore, a matter of interest to record what seem to be unquestionable reactions dependent upon the olfactory apparatus of our common freshwater catfish, Amiurus nebulosus.

Amiurus nebulosus is a bottom-feeding fish possessing fair powers of sight and unusual gustatory organs located not only in the mouth and on the general outer surface of the body, but especially on the eight barblets about the mouth (Herrick, '03). It is a hardy fish, living well in confinement and undergoing operations with success. It possesses near its anterior end a pair of nasal chambers each of which is provided with two apertures, one anterior, the other posterior. The anterior aperture is nearly circular in outline and is located on a slight conical elevation somewhat anterior to the root of the dorsal barblet. The posterior aperture is slit-like in form and lies immediately posterior to the same barblet. The anterior aperture is apparently always open; the posterior one seems capable of slight closure, but is usually freely open.

By keeping catfishes a few days without food, they can be made most eager for it, and if into an assemblage of such individuals, a few fragments of fresh earthworms are dropped, the excitement that ensues will last some time after the final piece of worm has been swallowed. During this period the fishes swim about excitedly in the lower part of the aquarium, now in this direction, now in that, and frequently sweep the bottom with their barblets. As can be noticed when the feeding actually occurs, the fishes seldom seize a fragment of worm till their barblets have come in contact with it. Yet before they have thus touched any food, they show a marked degree of excitement and it is this initial nervous state that would lead an observer to suspect that they scented their food.

I, therefore, took this phase of their activity as the one to be tested in connection with their olfactory apparatus.

A number of fishes that had been without food for several days were isolated in small vessels of water and, after an hour or more, when they had come to rest, they were tested with a solution filtered from a mixture of freshly chopped earthworms and tap-water. By means of a very fine glass pipette a small amount of this solution was discharged directly over the anterior olfactor aperture of a given fish and the fish was then closely watched. Notwithstanding the most careful manipulation, more or less of this solution could be seen at times to be swept into the mouth of the fish by the respiratory current and may well have stimulated the gustatory organs in that cavity. The subsequent movements of the fish were extremely irregular, and, though I was reasonably sure that as a result of the application of the solution the fishes respired more deeply and fully than before, I could not be certain that this reaction was not due to oral stimulation. Though I could see that some of the solution applied to the nasal aperture was sucked into the mouth, I was unable to make out whether any of it really entered the nasal chamber itself. As it is essential for the stimulation of the olfactory surfaces that the exciting material shall make its way to them, I turned next to the accessibility of these surfaces from the exterior.

The nasal apertures of the catfish are apparently always open and when a fish is swimming with some vigor through the water its motion doubtless drives a current of water through each nasal chamber. I tried to demonstrate this current indirectly by making a fish swim for five minutes through water containing a small amount of starch in suspension and then comparing the contents of its nasal chambers with that from the chambers of a fish that had been held motionless for a like period of time in the same water. So far as the comparison was concerned, the results were inconclusive, but the microscopic examination of the freshly opened nasal chambers led to the discovery that they were lined with cilia which were beating vigorously and persistently.

To ascertain whether these cilia produced a current of water through the nasal chambers, a freshly prepared fish-head was

immersed in water and the nasal apertures were tested with a mixture of water and carmine. By this means it was easy to demonstrate that a current of water entered the anterior nasal aperture and emerge from the posterior one, as has already been shown in Amia by Brookover ('10, p. 77), and that the water passed through the olfactory chamber of the catfish in from eight to ten seconds. It is therefore certain that even in the resting fish a continuous current of water is coursing through the nasal chambers from anterior to posterior, and, judging from the position of the nasal apertures on the body of the fish, this current is probably accentuated by forward locomotion.

If the nasal chambers of a resting fish are continuously provided with a flow of water from the exterior by which odorous material may be carried to the olfactory surfaces, the failure of the animal to respond to such material must have some deeper seat than the receptive organ. Since what appeared in the normal catfish to be a scenting reaction was observed only when the animal was in locomotion, it seemed to me that this condition might be the only one under which olfactory responses would be exhibited, and that the resting fish represented a state wholly unfavorable for such reactions. In other words, it seemed possible that only when the central nervous organs were discharging impulses to locomotion were they in a condition to transmit impulses eminating from the olfactory receptors. With this idea in mind, I set about devising a new line of experimentation to be carried out on the actively swimming fish.

As a preliminary to a revised method of procedure, five normal fishes were placed in a large aquarium over night that they might become accustomed to their surroundings. In this aquarium were then hung two wads of cheese-cloth, in one of which was concealed some minced earthworm. The fishes, which were swimming about near these wads, were then watched for an hour and their reactions in reference to the wads were recorded. The wad without worms was passed by the fishes many times and did not excite any noticeable reaction. The wad containing the worms was seized and tugged at eleven times in the course of the hour notwithstanding the fact that from time to time this

and the other wad were interchanged in position. Not only did the fishes thus openly seize this wad, but, when in its neighborhood, they would often turn sharply as though seeking something but without success, a form of reaction seldom observed near the wad which contained no worms. Two other sets, of five normal fishes, each, were tested in this manner and with similar results. It was perfectly clear to anyone watching these reactions that the fishes sensed the difference between the wad of cloth with worms and that without worms.

To ascertain what receptive organs were concerned in the reactions just described, I took from among the fifteen normal fishes already tested two sets of five each and prepared each set differently by subjecting its members to a special operation. One set was etherized, and, through a small incision between the eyes, their olfactory tracts were cut thus rendering their peripheral olfactory apparatus functionless. From fishes of the other set all the barblets were removed whereby their external gustatory organs were partly, though not wholly, eliminated. After these operations both sets of fishes were liberated in the large aquarium where they remained for over two days. At the expiration of this time, they were carefully inspected and tested. They swam about in an essentially normal way and members of both sets snapped bits of worm from the end of a hooked wire much as a normal fish does. I therefore judged them to be in a satisfactory condition for experimentation.

The tests were begun by introducing into the large aquarium containing the ten fishes a wad of cheese-cloth within which were hidden some minced earthworms and recording the kind of fish that visited it and the nature of their reactions. During the first hour the wad was seized 34 times by fishes without barblets but with normal olfactory organs and, though often passed by fishes with cut olfactory tracts, it was "nosed" only once by one of these. I next substituted a wad of cheese-cloth without worms for that with worms and recorded the reactions of the fishes for a second hour. Though members of both sets frequently swam by this wad, none at any time during the hour seized it or even nosed it. These tests were repeated on the same fishes for two suc-

ceeding days and with essentially similar results. On the second day the wad with worms was seized 16 times during the test hour by fishes with normal olfactory organs and on the third day 54 times. On both these days the fishes with their olfactory tracts cut made no attempts on the wad with worms nor did any fish at any time nose the wormless wad. The movements of the two sets of fishes when in the neighborhood of the wad containing minced worms were characteristically different. The fishes with their olfactory tracts cut swam by the wads without noticeable change; those without barblets, but with their olfactory apparatus intact almost always made several sharp turns when near the wad as though seeking something, and then either moved slowly away or swam more or less directly to the wad and began to nose and nibble it. These reactions were so clear and so characteristic that when taken in connection with the conditions of the fishes, they lead inevitably to the conclusion that the olfactory apparatus of the catfish is serviceable in sensing food at a distance much beyond that at which the organs of taste are capable of acting; in other words, catfishes truly scent their food.

Whether such olfactory reactions as those that have just been described are really due to smell or not is regarded by some authors as an open question. Nagel ('94, p. 56), who has discussed this matter at some length, concluded on rather theoretic grounds that fishes could not possibly possess a sense of smell and that their so-called olfactory organs act more as organs of taste than of smell. Possibly the whole matter is merely one of definition. With human beings smell differs from taste chiefly in the concentration of the stimulating solution and not, as was formerly supposed, on the state of the stimulating material, for, though we usually say that we smell gaseous or vaporous materials and taste liquids and solids, all these substances are in reality dissolved on the moist surfaces of which ever sense organ they stimulate. The most striking difference between taste and smell with us is that we smell extremely dilute solutions and taste only very much more concentrated ones. As a result we recognize the presence of many distant bodies by smell and not by taste, for the very minute amount of material that reaches us from the dis-

tant body will form a solution on our moist surfaces that will be stimulating for our organs of smell but not for our organs of taste. Hence our olfactory organs as compared with our organs of taste are what Sherrington ('06) has called distance receptors, a designation justly emphasized by Herrick ('08). Although this distinction between taste and smell is one of degree rather than of kind, it seems to me reasonably sound and it certainly holds in the case of the catfish much as it does with us, for this fish responds through its olfactory organs to solutions too dilute to affect its gustatory organs, and the nature of the response to olfactory stimulation (seeking food, etc.) is such that the olfactory organ in this fish can be called appropriately a distance receptor. I therefore believe that the catfish, though a water-inhabiting animal, possesses an olfactory organ that is as much an organ of smell as is the olfactory organ of the air-inhabiting vertebrates.

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